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PLANT PHYSIOLOGY

APRIL, 1930

ASH CONSTITUENTS OF PASTURE GRASSES, THEIR STANDARD ELECTRODE POTENTIALS AND ECOLOGICAL SIGNIFICANCE

H. P. Cooper

Since there is a wide variation in the ash constituents of a species of plant grown under various conditions, it was decided to determine the ash constituents of several pasture grasses grown on soils differing widely in productivity. An examination of some available data on ash analyses of pasture plants suggested that the only way to secure definite information on this important topic was to attempt to determine, by correlating the existing data, the order of absorption and the condition necessary for optimum assimilation of the various nutrient ions.

The lack of a close correlation between the H-ion concentration of the soil and plant growth suggested a consideration of the potential relations which might result from the presence in the soil complexes of electropositive elements above and below hydrogen in the electromotive series. Such a consideration soon made it evident that the electromotive series could be used very effectively in correlating much of the data in the field of nutrition. It was observed that there is a direct relation between the order of removal of ions from soil colloids by electrodialysis, and the order of atomic ions in the electromotive series (**19, 20**). It was also observed that many organisms selectively absorb atomic nutrient ions in the same order as they appear in the electromotive series. Observations on the selective absorption of the strong ions led to a consideration of the radiant energy necessary for the optimum assimilation of the various nutrient compounds. A consideration of thermo-dynamical data clearly showed that the physiological effects of radiant energy are definitely related to the decompositional voltages, or processes in which a change of valence takes place, of nutrient materials. Since so much of the data on physiological problems can be correlated with the electromotive series it is very probable that this series

could be used more generally in the interpretation of data which have been obtained in biological investigations.

Selection of material for ash analysis

Many published analyses of pasture plants represent mixed herbage, including grasses and clovers. In such investigations it would probably be more desirable to select grass samples from situations where one grass predominated. Since there is a more or less definite succession of pasture plant associations accompanying the depletion of soils, it was decided to select samples from the four major associations found in New York pastures. Kentucky blue grass usually dominates on pastures which are in a high state of fertility, and as soon as the soils become depleted of the elements which are characteristic of a high state of fertility, Kentucky blue grass may be followed by Rhode Island bent grass or red top. On further depletion of the soil, sweet vernal grass may dominate, and finally poverty grass, weeds and trees may represent the dominant type of vegetation.

The samples from these four associations were taken when the grasses were in full bloom or shortly after. The Kentucky blue grass and sweet vernal grass samples were collected between the middle of June and the first of July. The Rhode Island bent grass and poverty grass samples were obtained three to four weeks later, since these do not bloom until relatively late in the season. Soil and subsoil samples were taken for hydrogen-ion determinations at the time the grass samples were collected.

Ash analyses of pasture grasses

The ash analyses of pasture grasses which were made in the Agronomy laboratory are given in table I. The data on Kentucky blue grass, Rhode Island bent grass, sweet vernal grass and poverty grass are from common pasture plant associations found in New York. The data on Canadian blue grass, broomsedge and rice cut-grass do not represent typical pasture conditions. These latter samples were selected to demonstrate the tolerance of plants to various ecological conditions.

The nitrogen and the ash content of the plants seem to decrease as the soils become depleted. Potassium oxide is seen to constitute from about one-third to two-fifths of the ash content of the common pasture grasses. It is relatively high in Kentucky blue grass and sweet vernal grass, but it is relatively low in Rhode Island bent grass and poverty grass. The calcium oxide content is highest in Rhode Island bent grass and poverty grass. This reverse relationship between the potassium and calcium contents seems to be related to the season of the most rapid growth period of these plants. The plants with their rapid growth period early in the season such as Kentucky blue and sweet vernal grasses are relatively high in potassium

TABLE I

NUTRIENT ELEMENTS IN THE ASH OF PASTURE GRASSES REPRESENTING THE VARIOUS PLANT ASSOCIATIONS CHARACTERISTIC OF THE DIFFERENT SOIL FERTILITY LEVELS FOUND IN NEW YORK

Grass	Number of sample	Average pH of soil	Per cent. K_2O		Per cent. CaO		Per cent. MgO		Per cent. P_2O_5		Per cent. SiO_2		N percentage		Ash
			Plant	Ash	Plant	Ash	Plant	Ash	Plant	Ash	Plant	Ash	Plant	Ash	Percentage
Kentucky blue (*Poa pratensis*)	9	6.79	2.36	39.07	0.18	2.98	0.18	2.98	0.57	9.44	1.91	31.62	1.51	25.00	6.04
Rhode Island bent (*Agrostis tenuis*)	12	5.18	1.44	25.90	0.33	5.93	0.20	3.60	0.39	7.01	2.55	45.86	1.33	23.92	5.56
Sweet vernal (*Anthoxanthum adoratum*)	6	5.28	1.84	41.34	0.16	3.59	0.12	2.69	0.41	9.21	1.15	25.84	1.11	29.94	4.45
Poverty (*Danthonia spicata*)	5	5.58	1.45	39.29	0.27	7.32	0.13	3.52	0.25	6.77	1.18	31.98	0.88	23.85	3.69
Canadian blue (*Poa compressa*)	4	7.64	1.18	32.67	0.28	7.76	0.14	3.88	0.22	6.09	1.34	37.12	0.75	20.77	3.61
Broomsedge (*Andropogon virginicus*)	1	4.74	0.40	7.78	0.19	3.70	0.05	0.97	0.30	5.84	4.21	81.91	0.71	13.81	5.14
Rice cut-grass (*Leersia oryzoides*)	2	5.17	0.51	5.12	0.21	2.11	0.17	1.72	0.43	4.32	8.15	81.91	1.18	11.86	9.95

and low in calcium while the plants with their rapid growth period late in the season are relatively high in calcium and low in potassium. The per cent. of phosphorus in the ash seems to be positively correlated with the potassium and negatively correlated with the calcium content. The silica content of the hard and unpalatable grasses such as Rhode Island bent grass, broomsedge and rice cut-grass is relatively high.

The correlations between the various constituents are given in table II. They represent the percentages of the entire plant with the exception of the correlation -0.45 ± 0.08 for P_2O_5 and SiO_2 which represents the percentage of these elements in the ash of the plants.

TABLE II

CORRELATION COEFFICIENT FOR THE NITROGEN AND ASH CONTENT OF FORTY-FIVE SAMPLES OF PASTURE GRASSES

K_2O and CaO	-0.87 ± 0.023
K_2O and MgO	0.17 ± 0.11
K_2O and P_2O_5	0.76 ± 0.043
K_2O and N	0.76 ± 0.043
K_2O and ash	0.65 ± 0.06
CaO and MgO	0.45 ± 0.08
CaO and P_2O_5	-0.44 ± 0.08
CaO and N	0.001
CaO and ash	-0.37 ± 0.09
P_2O_5 and N	0.84 ± 0.03
P_2O_5 and SiO_2*	-0.45 ± 0.08
P_2O_5 and ash	0.64 ± 0.06

* Represents percentages of ash rather than percentages of plant.

Significance of data

A book by ORR (**46**) contains an excellent summary of the data on the mineral content of pasture plants, therefore only data of particular interest are cited. In previous papers COOPER, *et. al.* (**19, 20**) have discussed some of the electro-chemical factors influencing the nutrition of organisms. It was pointed out that there is a correlation between the position of ions in the electromotive series and their activity in the soil collodial complexes. There is also a relation between the order of ion absorption by many organisms and the potential series. The strength of ions normally utilized by organisms may also affect the quality of radiant energy necessary for optimum growth.

An inspection of table I clearly indicates that the strong ions are absorbed in greater quantities than the weaker ones and that the metallic ash constituents are in a more or less definite order. This is in agreement with calculations made by the author from data on the ash analyses of

forty-eight plants reported by ROBINSON, *et. al.* (**52**). These show the order of constituents as follows: K_2O 34.36 per cent., CaO 19.63 per cent., MgO 7.13 per cent., Al_2O_3 1.34 per cent., Fe_2O_3 0.61 per cent., P_2O_5 9.87 per cent., SiO_2 9.86 per cent., SO_3 8.98 per cent., and Cl_2 5.49 per cent. The metallic elements are in the same order as their standard electrode potentials. ASTON (**5, 6, 7**) reports data on the constituents of 70 samples of pasture plants which include samples from regions of New Zealand where iron is deficient in forage plants. His average data for 47–70 samples are as follows: CaO 1.05 per cent., MgO 0.47 per cent., Al_2O_3 0.13 per cent., Mn_3O_4 0.045 per cent. and Fe_2O_3 0.041 per cent. ELVEHJEM and HART (**22**) reported 199 mg., 65.8 mg., and 13.5 mg. per kilogram of dry matter respectively for iron, manganese and copper in forty-seven feed analyses. All of these data suggest that many plants selectively absorb the strong ions or the elements with relatively high standard electrode potentials.

It is interesting to note that there is more Mn_3O_4 than Fe_2O_3 in the New Zealand forage plants. It was observed by RIGG and ASKEW (**51**) that on the poorer pastures the plants contained more manganese than iron, and were also characterized by their high content of silica. There does not seem to be a deficiency of iron in the soil but it is not absorbed by plants. Since the Mn ion is stronger than the Fe ion, manganese is apparently selectively absorbed and probably partially substituted for iron in the growth of the plant. The pasture plants grown on soils relatively high in available manganese may not contain enough iron for the maintenance of animals, since it is an active constituent of the blood. As iron is apparently not an active constituent of chlorophyll, elements which have similar physiological effects may partially substitute for it in the pasture plants.

Table I shows that potassium is the most abundant electropositive element in the ash of the plant analyzed. There is a relatively high percentage of K_2O in the ash of Kentucky blue grass and sweet vernal grass. It is lower in Rhode Island bent grass and poverty grass. The high K_2O content in the ash of Kentucky blue grass is in agreement with the response of this plant to potash fertilizers as reported by WHITE and HOLBEN (**67**) and WHITE and GARDNER (**68**). It is also noteworthy that there is 0.18 per cent. CaO in the ash of Kentucky blue grass and 0.33 per cent. CaO in Rhode Island bent grass. This is an interesting relationship because the Kentucky blue grass samples were taken mostly from limestone soils. The average pH value (6.79) indicates that the soils were well supplied with available calcium. Most of the Rhode Island bent grass samples were taken from non-limestone soils as is indicated by the average pH value of 5.18. These inverse relations between K_2O and CaO show that the ash constituents of plants are largely determined by the amounts of various constituents available in the soil.

The phosphorus and the nitrogen contents of Kentucky blue grass are also relatively high, which suggests that this plant requires a relatively high fertility level for its successful growth. Rhode Island bent grass is lower in phosphorus and nitrogen than Kentucky blue grass but it is higher in silica. These data suggest that Rhode Island bent grass will tolerate a lower fertility level than will Kentucky blue grass. The ash and nitrogen content decrease from Kentucky blue grass to poverty grass, which is a good measure of the relative tolerance of these plants to various soil fertility levels. The broomsedge and rice cut-grass are extremely high in silica and low in constituents which form strong ions. These two plants are very tolerant of shade. Since these plants utilize large amounts of weak electrolytes they should be capable of tolerating a low intensity and low quality of light.

Correlations between the various constituents of pasture grasses are given in table II. There is a negative correlation of -0.87 ± 0.023 between K_2O and CaO; a negative correlation of -0.44 ± 0.08 between CaO and P_2O_5; and a negative correlation of -0.45 ± 0.08 between the P_2O_5 and SiO_2. The correlation coefficients for the other constituents are positive or not significant. There is a high positive correlation between P_2O_5 and N. This might be expected in case either of these is a limiting factor in plant growth. These two nutrients are in the same family of elements; therefore, they would be expected to have similar functions in the nutrition of organisms. With certain legumes and where liberal amounts of available phosphorus and nitrogen are present a negative correlation may exist between the amounts of these materials in the plant. Legumes may often be relatively low in silicon and phosphorus since these plants have available to them nitrogen which forms relatively strong electro-negative combinations. ARCHIBALD and NELSON (**2**) present data showing that the phosphorus content of pasture plants was about 10 per cent. higher on unfertilized check plats than on plats liberally fertilized with a complete fertilizer and calcium nitrate.

Data presented by SCOTT (**55**) relating to the phosphorus deficiency in forage plants is of special interest in this connection. He found as an average of nine samples of alfalfa 2.39 per cent. CaO and 0.249 per cent. P_2O_5 or a ratio of Ca : P of 15.8 : 1 which is much wider than the ratios by which rickets have been produced experimentally in certain small animals. This suggests that plants can tolerate a wider range in the Ca : P ratio than do certain animals. The phosphorus content was not particularly low in the soil from which the alfalfa was taken but the element was not available to the plants. Where the Ca : P ratio is as wide as observed in these samples the phosphorus usually exists in stable combinations and its assimilation would probably require short wave lengths of light.

A negative correlation of -0.44 ± 0.08 was obtained between CaO and P_2O_5. Calculations made from the ash data of forty-eight plants published by ROBINSON, STEINKOENIG and MILLER (**52**) show a negative correlation of -0.51 ± 0.08 between CaO and P_2O_5. These data also show a negative correlation of -0.70 ± 0.055 between the sum K_2O, Na_2O and CaO, and the sum of SiO_2, P_2O_5, Cl_2 and SO_3. These negative correlations suggest that in soil relatively high in available strong basic materials the hydroxyl, bicarbonate, and carbonate ions may be absorbed by plants in relatively large amounts. The relatively weak hydroxyl and bicarbonate ions form compounds which are relatively unstable as compared to strong sulphate, phosphate and fluoride ions which form relatively stable compounds and require for optimum assimilation relatively short wave lengths of radiant energy.

The strong negative correlation between K_2O and CaO is in agreement with the finding of FONDER (**24**) on the K_2O and CaO content of alfalfa grown on various soils.

Some factors influencing the ash constituents of plants

Standard electrode potentials are quantitative values which are very useful in correlating data. Since the standard electrode potentials include the heat of solvation of ions, some of the low atomic weight elements, which have highly hydrated ions, may be out of the order of their replacement value. In case of the alkali metals the ionization potentials give a better ion series than the standard electrode potentials.

MICHAELIS (**43**) has shown that there is a close correlation between the order of ion adsorption by blood charcoal and the electromotive tension series; the stronger the ion the less the adsorption by blood charcoal.

Data on base exchange by GEDROIZ (**25**) and numerous others may be interpreted as showing that there is a correlation between the order of atomic ions in the electromotive series and their activity in the soil colloidal complexes. Data published by WILSON (**70**) and MATTSON (**39**) on the liberation of ions from soil colloids by electrodialysis are of particular interest in this connection. Their data may also be interpreted as showing that there is a close correlation between the order of elements in the potential series and the order of removal of cations from soil colloids by electrodialysis.

It is not probable that large amounts of atomic ions below calcium in the electromotive series with the exception of hydrogen will be found in the soil colloidal complex. The calcium ion is the lowest metallic atomic ion in the potential series which would be expected to exist in large amounts in soil colloidal complexes. The H-ion often ranks next to calcium in its activity in soil colloids. The activity of the H-ion in soil colloids is greater

than might be expected from its position in the potential series. The ionization potentials of elements in various stages of ionization presented by MILLIKAN and BOWEN (**44**) and NOYES and BECKMAN (**45**) suggest the explanation for the relative abundance of the H-ion in colloidal complexes of certain acid soils. The last normal valence electron in the nutrient elements below calcium in the potential series is more strongly bound than the single valence electron of hydrogen. Approximately 6.07 equivalent volts are required to remove the last bound electron in calcium, and 11.74 volts for the second valence electron of calcium. With magnesium, the next element below calcium in the potential series, the energy of removal for the first and second electrons is 7.56 and 14.98 equivalent volts respectively, whereas the energy required to remove the valence electron of hydrogen is approximately 13.5 equivalent volts.

Since the H-ion often ranks next to calcium in its activity in soil colloids large amounts of electropositive elements below calcium in the electromotive series would not be expected in the ash of plants. Since the ionization potential of hydrogen and magnesium are so near together it might be expected that approximately the same quanta of radiant energy would be required for their optimum assimilation. It is interesting to note that magnesium is essential for the formation of fats and oils. Where the soil colloids are relatively high in H-ions and Mg-ions the conditions are often favorable for the dominance of resinous plants such as coniferous and gum trees. Complex ions such as NH_4-ion may often be an important nutrient ion particularly on acid soils. It is apparently readily assimilated by certain plants.

Data on permeability have been assembled by STILES (**63**) and they suggest that there is a relationship between the order of absorption and the strength of ions. As previously noted, it should not be overlooked that the low atomic number elements, which may have highly hydrated ions such as lithium, in an ion absorption series may not be in agreement with standard electrode potentials.

Some of the most interesting and convincing data on the order of absorption of salts or ions are those reported by HOAGLAND and DAVIS (**30**), OSTERHOUT (**48**), and COOPER and BLINK (**18**). These very significant data may be interpreted to indicate that there is a very close relation between the strength of atomic ions and the absorption of electropositive elements. The data reported by COOPER and BLINK (**18**) suggest that certain organisms may absorb isosteric ions. *Halicystis* seems to selectively absorb neon-like cations while species of *Valonia* selectively absorb argon-like cations from sea water. Membranes may act as ionic or molecular sieves according to MCBAIN and KISTER (**40**). Many organisms seem to be able to exclude some of the large strong ions, such as the xenon-like and

krypton-like cations. In considering the influence of dimensions of ions on the rate of penetration, the radii of hydrated as well as of unhydrated ions may be important. The conflicting, and probably much erroneous, data on permeability of ions make it very difficult to formulate a general statement concerning the order of penetration of materials. The best available data certainly suggest that many organisms tend to differentially absorb atomic ions in the same order as they appear in the electromotive series. Since the nature or porosity of membranes, the magnitude of the hydrated and unhydrated radii of ions, and the utilization of ions by organisms may greatly influence the order of absorption of materials, it is difficult to establish an absorption series for various membranes.

Relation of mineral content of plants to shade tolerance and feeding value

It is logical to assume (and the results from experiments seem to justify the assumption), that pasture plants grown on soils where the complex is favorable for autotrophic bacteria and the chemosynthetic production of carbohydrates, such as occurs in the nitrification and sulphofication processes, have a relatively high feeding value. Chemosynthesis is stimulated by the presence of metallic elements with relatively high standard electrode potentials such as K, Na and Ca. The free energy decrease in the formation of certain compounds of these elements is sufficiently large to satisfy the energy requirements of the autophytic soil organisms. BAAS-BECKING and PARKS (**8**) have summarized some of the data on the energy requirements of autophytic bacteria. BUCHANAN and FULMER (**14**) have also compiled data on the energy requirements of certain organisms. The utilization of the relatively stable nutrient compounds by crop plants will probably require relatively large quanta of light energy and may result in the formation of organic compounds of high feeding value. Plants which require a fertile soil, or strong ions, are not very tolerant of shade and usually have a high feeding value.

Since the discovery was made that light having a wave length between 3130 and 2900 Ångstroms or approximately 3.94 to 4.26 equivalent volts protect animals from rickets, there has been an interest in securing foods which may supply equivalent quanta of energy. The researches of STEENBOCK and BLACK (**62**), HESS and WEINSTOCK (**29**), KNUDSON and MOORE (**36**), LAURENS (**37, 38**) and numerous others have shown that the radiation which renders foods active biologically is similar in wave lengths to that which protects animals against rickets when they are directly exposed to the rays. SHOHL, BENNETT and WEED (**58**) and others have shown that the acid-base content of the diet is an important factor in either the production or the cure of experimentally induced rickets. These

experimental results suggest that in certain cases the stability of the calcium and phosphorous compounds available to the organism is an important factor. The data in tables III and IV indicate that the less stable forms of phosphorus, such as H_3PO_4, are much more easily assimilated than the more stable forms such as $Ca_2H_2(PO_4)_2$ or $Ca_3(PO_4)_2$. The latter two compounds very probably require relatively larger quanta of energy for their assimilation than do the less stable combinations.

Physiological effects of radiant energy

Since many organisms selectively absorb the strong ions, it is necessary to consider the quality of radiant energy required for the optimum utilization of various nutrient ions. HOAGLAND, DAVIS and HIBBARD (**31**) and TOTTINGHAM and LOWSMA (**65**) have found that light effects the intake of certain nutrient ions by plants.

Table III gives the approximate energy corresponding to various wave lengths of radiation in gram-calories and volt-Faradays. At sea-level the

TABLE III

APPROXIMATE EQUIVALENT ENERGY CORRESPONDING TO VARIOUS WAVE LENGTHS OF LIGHT IN GRAM-CALORIES AND VOLT-FARADAYS

COLOR OF LIGHT	WAVE LENGTH IN ÅNGSTROMS	ENERGY	
		GRAM-CALORIES	VOLT-FARADAYS
Infra-red	30000–7500	9,432– 37,800	0.41–1.64
Red	7500–6500	37,800– 43,630	1.64–1.89
Orange	6500–5900	43,630– 48,060	1.89–2.08
Yellow	5900–5750	48,060– 49,320	2.08–2.14
Green	5750–4900	49,320– 57,880	2.14–2.51
Blue	4900–4550	57,880– 62,330	2.51–2.70
Violet	4550–3950	62,330– 71,800	2.70–3.11
Ultra-violet	3950–2900	71,800– 97,900	3.11–4.26
Ultra-violet*	2900–2000	97,900–142,000	4.26–6.16

* Artificial, a very low intensity or none in the solar spectrum.

short wave length limit of the solar spectrum is around 2900 Ångstroms. Each wave length has a definite energy value. The shorter the wave length of radiation, the larger the quanta of radiant energy. The size of quanta in the shortest wave lengths of the solar spectrum is about two to two and one-half times the size of the quanta in red radiation.

As living matter is a reduced system, radiant energy may be effective in nutrition (1) by decomposing nutrient compounds, (2) by causing a change of valence of certain elements and (3) by activating certain molecules, atoms, or ions. The resonance radiation from activated materials may be

an important factor in the catalytic activity of certain materials. Therefore the energy necessary for the decomposition or the reduction of nutrients and the resonance radiation from activated materials are of special interest in nutritional studies.

It is possible to get the approximate relative minimum energy of decomposition of certain simple compounds from their standard electrode potentials (**20**). The approximate relative energy of formation in volt-Faradays of various chlorides, calculated from standard electrode potentials and the equivalent quality of light energy, are given in table IV. Radiant energy equivalent to or greater than the free-energy decrease in the formation of simple electrolytes may be necessary for their optimum assimilation by autophytic plants. Elements which cannot be readily reduced by the radiant energy characteristic of the solar spectrum are not found as actual constituents of many organic compounds.

TABLE IV

APPROXIMATE RELATIVE HEAT OR ENERGY OF FORMATION, CALCULATED FROM ELECTRODE POTENTIALS, OF SOME NUTRIENT ELECTROLYTES AND THE CORRESPONDING EQUIVALENT ENERGY VALUE IN LIGHT

ELECTROLYTE	ENERGY OF FORMATION IN VOLT–FARADAYS	EQUIVALENT LIGHT ENERGY
$CuCl_2$	1.01	infra red
HCl	1.35	infra red
$NiCl_2$	1.57	infra red
$CoCl_2$	1.64	red
$FeCl_2$	1.78	red
NH_4Cl	1.90	orange
$CrCl_2$	1.95	orange
$ZnCl_2$	2.11	yellow
$MnCl_2$	2.45	green
$AlCl_3$	2.65	blue
$MgCl_2$	2.85	violet
$CaCl_2$	3.85	ultra-violet
$NaCl$	4.05	ultra-violet
KCl	4.28	ultra-violet
H_3PO_4	1.70*	red
$Ca(OH)_2$	2.91	violet
$Ca_2H_2(PO_4)_2$	4.20*	ultra-violet
CaF_2	4.46	ultra-violet

* Calculated from discharge potential of acid.

The relative equivalent energy of formation of $FeCl_2$ corresponds to the equivalent radiant energy in red light. The corresponding value for $MgCl_2$

is equivalent to the energy of violet light. ARTHUR (3) has shown that the elimination of either the red or blue portion of the spectrum greatly affects the growth of certain plants. SHULL (59) has called attention to the selective absorption of light by leaves in the red and blue violet portion of the spectrum. It was observed by SAYRE (53) that wave lengths of radiant energy longer than 6800 Ångstroms, which is equal to 1.81 equivalent volts, are not effective in the formation of chlorophyll in seedlings. This value is very near the decomposition voltage of $FeCl_2$ which is 1.78 volts. The energy for the reduction of BO_3^{---} to BO_2^- is also 1.78 equivalent volts. This may indicate that certain iron and boron compounds selectively absorb similar quanta of radiant energy.

Chlorophyll "a" has a strong absorption band around 6800–6370 Ångstroms, equal to 1.87 to 1.84 equivalent volts. It has another strong absorption band around 4300–4400 Ångstroms, equal to 2.87 to 2.80 equivalent volts. These values are in close agreement with the decomposition voltage of $MgCl_2$ and the reduction of HCO_3^- to HCO_2^- which is 2.85 volts. This indicates that these two materials selectively absorb similar quanta of radiant energy. Some of the less intense absorption bands of chlorophyll are in close agreement with the decomposition voltages of certain compounds of elements absorbed by plants as shown in table IV. There is apparently selective absorption of radiant energy around the critical decomposition potential of certain nutrient materials. It seems as if growth of plants is intimately related to the absorption of radiant energy approximately equivalent to the decomposition or reduction energy of certain nutrient compounds. A paper on the relation of nutrients to the formation of chloroplast pigments by SCHERTZ (54) is of special interest in this connection. SPOEHR (61) has presented a comprehensive discussion of this general topic.

The relation of decomposition voltage to nutrition is nicely demonstrated in the prevention or curing of experimental rickets by ultraviolet radiation. Rickets induced by a high calcium and a low phosphorus diet can be cured by exposing animals to light wave lengths between 3130 and 2900 Ångstroms which is equal to 3.94 to 4.26 equivalent volts. Maximum activity is from 3020 to 2970 Ångstroms or 4.08 to 4.17 volts according to COBLENTZ and STAIR (16). The decomposition voltage of calcium phosphate as calculated from standard electrode potentials of Ca^{++} (2.50 volts) and the discharge potential of $H_2PO_4^-$ (1.70 volts) is 4.20 volt-Faradays, which is equivalent to the energy in ultraviolet light as shown in tables III and IV. This suggests that the prevention of rickets in animals utilizing calcium phosphate may require radiant energy approximately equivalent to the decomposition voltage of calcium phosphate. It is suggested by SHEAR,

WASHBURN and KRAMER (**56**) that bones may be composed of $CaHPO_4$ instead of $Ca_3(PO_4)_2$. CLARK (**15**) found that ultraviolet light decomposed compounds of calcium and protein, and increased the ionic calcium in blood serum. Food stuffs which supply quanta of energy necessary for decomposition of calcium compounds may also be effective in preventing rickets. The decomposition voltage for H_3PO_4 is 1.70 volts, which is equivalent to the energy in red light. The relative energy of formation of $Ca(OH)_2$ is 2.91 volts or equivalent to the energy in violet light. These data on the energy of formation of compounds suggest that rickets may in certain cases be prevented or cured by supplying the animal, either in food or in radiant energy, the quanta of energy equivalent to the decomposition voltage of the calcium and phosphorus compounds being utilized by the animal (**56**). The data presented by SHOHL, BENNETT and WEED (**58**) definitely suggest that where calcium and phosphorus are available in less stable combinations than calcium phosphate, relatively small quanta of radiant energy may produce normal development.

Calcium phosphate is light stable to near the extreme short wave length limit of the solar spectrum. It would not be expected to be light stable beyond the short wave length of the solar spectrum. The only other common nutrient calcium compounds which would be expected to be more light stable than calcium phosphate are compounds of calcium and fluorine as shown in tables III and IV. The enamel of teeth contains considerable fluorine which makes it very light stable, probably not being affected until the quanta of radiant energy are much larger than is characteristic of the solar spectrum. The relation of fluorine to the development of teeth has been discussed by MCCOLLUM, SIMONDS and BECKER (**42**). Since considerable quantities of fluorine are found in many phosphate materials which are used as plant nutrients, fluorine may be present in many plants.

It is probable that many of the reactions caused by the absorption of radiant energy are due to oxidations and reductions or processes in which a change of valence takes place, with the liberation of O_2 from the reduced compound. The approximate equivalent minimum radiant energy required for the reduction of some common nutrient anions is given in table V (**32**). An inspection of this table shows that there is a great difference in the stability of the common nutrient anions. It is noted that the NO_3^- may be reduced by infra red, BO_3^{---} by red, HSO_4^- by blue, HCO_3^- by violet and $H_2PO_4^-$ by ultraviolet radiation. These data definitely suggest the reason why many plants respond so readily to nitrate nitrogen, particularly in nutrient solutions containing strong cations. The marked response of certain plants, particularly legumes, to boron compounds as reported by BRENCHLEY (**12**), SOMMER and LIPMAN (**60**), COLLINS (**17**), JOHNSTON and

DORE (**33**), may be definitely related to the small quanta of energy required for the reduction of borates. The sulphate and carbonate ions require a large quantum of energy for their reduction, while the phosphate ion is relatively stable and requires ultraviolet light for reduction. HARRIS (**27**) has noted that there is a group of light waves equal to 2.83–4.24 equivalent volts which has a marked effect upon the metabolism of rats and mice; an increase of 20 per cent. in CO_2 output was observed. These rays are very close to the minimum energy, 2.85 equivalent volts, necessary for the reduction of the HCO_3-ion which would probably result in the splitting off of O_2 and CO_2.

TABLE V

EQUIVALENT MINIMUM RADIANT ENERGY REQUIRED FOR THE REDUCTION OF SOME NUTRIENT ANIONS

NO_3^- + infra red radiation	11757 Å = NO_2^- + 1/2 O_2
BO_4^{---} + red radiation	6935 Å = BO_2^- + 1/2 O_2
HSO_4^- + blue radiation	4572 Å = HSO_3^- + 1/2 O_2
HCO_3^- + violet radiation	4332 Å = HCO_2^- + 1/2 O_2
$H_2PO_4^-$ + ultra-violet radiation	3685 Å = $H_2PO_3^-$ + 1/2 O_2
$H_2PO_3^-$ + ultra-violet radiation	3259 Å = $H_2PO_2^-$ + 1/2 O_2

The selective absorption of strong ions and of relatively stable compounds may limit or exclude the absorption of essential light sensitive elements, which have relatively low standard electrode potentials, such as iron, manganese and copper. The strength of ions which are endured by plants may influence their shade tolerance and palatability. In general the plants which grow well on relatively poor soil are more likely to be tolerant of shade, since they probably utilize mostly relatively weak ions. While the plants which require fertile soils and strong ions usually have a high feeding value.

The above mentioned relationships clearly indicate that there is a close relation between the heat, or energy, of formation and the energy required for decomposition, or the reduction of various nutrient compounds, and the quality of light necessary for their optimum assimilation by autophytic plants. The very interesting and significant work on the physiological effect of light at the Boyce Thompson Institute for Plant Research reported by ARTHUR (**3**), ARTHUR and NEWELL (**4**), POPP (**49**) and SHIRLEY (**57**) are of particular interest in this connection. Their work shows that plants respond differently to various intensities and qualities of light. SHIRLEY (**57**) found that redwoods and loblolly pine survive under a low light intensity. Also certain coniferous seedlings were found by BATES and ROESER (**9**) to tolerate very low light intensities. These relations might

be expected since such plants grow successfully on relatively poor soils and are tolerant of weak nutrients ions. But the sunflower plants which make their optimum growth on fertile soils and are very tolerant of strong ions require higher light intensity for optimum growth. The work of POPP (**49**) indicates that tomatoes and soybeans do not require for optimum growth as short wave lengths of light as do sunflowers and sudan grass.

The bimodal growth or production curve often observed in biological material may be related to the stability or sensitivity to solar radiation of the available nutrient compounds. Adsorption potentials and diffusion coefficients as calculated from mobilities of ions may also be important factors in determining bimodal growth curves.

Substitution of one nutrient element for another within a family or group of elements

The selective absorption of certain nutrient materials with relatively high standard electrode potentials indicates that within certain limits organisms may absorb materials with a positive or a negative charge of electricity rather than any specific ion. Therefore the ash constituents of a plant are largely determined by the available nutrients in the soil. This makes it necessary to consider groups of possible nutrient elements rather than any single ion. Since families of elements are qualitatively alike their chief differences are largely quantitative in character. Therefore one member of a family of elements may partially substitute for another in the nutrition of organisms. BREAZEALE (**11**) found that certain nutrients effect the absorption of others.

Elements may substitute for one another in nutrition in several ways (1) by substituting as an actual constituent of a specific organic compound, (2) in oxidation reduction reactions and (3) as catalytic agents. The possibilities of substitution in the two latter cases are probably much greater than in the first.

In the alkali metal family considerable quantities of Rb, K, Na and Li may be found in various plants. Certain plants can apparently utilize Rb for a certain quantity of K. More Rb than K was found in the growing points of cereals by RAMAGE (**50**). Certain plants seem to get along quite as well with Na as with K. A large amount of Li is toxic to many plants. There is often an inverse relation between the Mg, Ca, and Sr in organisms. Some relations between calcium and strontium in nutrition have been discussed by WHEELER (**66**), and KINNEY and MCCOLLUM (**35**). HAAS (**26**) reports the inverse relation between the content of Ca and Mg in plants and their relation to chlorosis.

In the halogen family the elements differ widely in their electrical properties. Fluorine is the most electronegative element and its compounds are relatively light stable, while iodine forms unstable compounds. Iodine compounds are relatively easly decomposed by radiant energy which is characteristic of the solar spectrum. It is highly probable that the marked contrast in the physiological effect of F and I is related to their sensitivity to radiant energy. McClendon (**41**), and Orr and Leitch (**47**) have discussed extensively the relation of iodine to nutrition. Chlorine and bromine are intermediate between F and I in their properties, and there is apparently a rather wide range in the substitution of Br for Cl with certain organisms, according to data presented by Hoagland, Davis and Hibbard (**31**).

Nitrogen and phosphorus are in the same family of elements and they undoubtedly have many similar functions in the nutrition of organisms. A liberal quantity of one of these elements may enable an organism to function normally with a minimum quantity of the other. The works of Clark (**15**) and Csapo (**21**) suggest that under certain conditions a high blood protein content may interfere with the precipitation of calcium phosphate and normal bone formation. It is suggested that ultraviolet radiation may decompose the compounds of calcium and protein and increase the ionized Ca in the blood.

In oxidation-reduction reactions there is a possibility of a rather wide range of substitution. Nitrates, borates, sulphates, carbonates and phosphates may undergo reduction by the absorption of radiant energy characteristic of the solar spectrum.

Many of the elements which act as catalysts are found in the transition series in the periodic chart of the elements. The color of ions and the catalytic properties of elements in the transition series may be definitely related to the comparatively small energy difference between any two states in which valence electrons may exist. The resonance radiation from these easily activited elements may be an important factor in determining the catalytic activity of certain materials. Similar responses may be expected from elements with atomic numbers from 21 to 30 including Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu and Zn. It has been observed by Felix (**23**), Allison, Bryan and Hunter (**1**), Bryan (**13**), Willis (**69**), that certain of these constituents are effective in increasing plant growth, particularly on organic soils relatively high in strong cations. The researches of Hart, Steenbock, Waddell and Elvehjem (**28**), Titus, Cave, and Hughes (**64**) and Beard, Myers and Shipley (**10**) have shown that such materials as Mn, Fe and Cu may have similar or supplementary effects in the nutrition of animals. It is possible that similar physiological responses might be

produced by some of the transition elements in the fifth and sixth periods of the chart of the atoms.

Summary and conclusions

1. Ash analyses of pasture grasses, selected from the four major associations found in New York pastures, are presented.

2. These analyses show that there is a close correlation between the standard electrode potentials of elements and the amount of the various minerals in the ash of plants.

3. The samples taken from productive soils contained relatively large amounts of those elements which form strong ions, while the samples taken from unproductive soil contained a relatively low ash content and were relatively high in the elements which form weak ions.

4. A negative correlation exists between K_2O and CaO and between CaO an P_2O_5 in the ash of plants. Calcium seems to be negatively correlated with the strong anionic materials which suggest that plants growing on alkaline soils may absorb considerable quantities of the hydroxyl and bicarbonate ions.

5. There seems to be a relation between the strength of ions a plant will endure and its shade tolerance and feeding value. Plants which make their optimum growth on fertile soils and require strong ions are not very tolerant of shade and are of high feeding value, whereas plants which grow on poor soils and endure relatively large quantities of weak ions are more shade tolerant and usually have a lower feeding value.

6. Radiant energy may be effective in nutrition (1) by decomposing nutrient compounds, (2) by reducing or causing a change of valence of certain material and (3) by activating certain atoms or ions, which may emit resonance radiation. The approximate relative free energy decrease in the formation of certain simple electrolytes may be secured from standard electrode potentials.

7. The results of recent experiments by numerous investigators indicate that radiant energy equivalent to the decomposition voltage seems to greatly facilitate the assimilation of calcium phosphate. This is nicely demonstrated by the effect of ultraviolet light in the prevention or cure of rickets.

Since within certain limits organisms appear to absorb materials with a positive or negative charge rather than any specific ion, it is possible that one element may substitute for another within a family or group of elements. The extent of the possible substitution will probably vary with the organism and the properties of the elements involved.

Many catalytic agents are in the transition series of the periodic chart of the atoms. The color of ions and the catalytic properties of elements

in the transition series may be definitely related to the comparatively small energy differences between any two states in which valence electrons may exist. The resonance radiation from easily activated materials may be an important factor in determining the catalytic activity of certain materials. The energy relations discussed in this paper prove very useful in interpreting some of the recent experimental results on the effect of radiant energy on organisms.

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PRELIMINARY RESULTS IN MEASURING THE HARDINESS OF PLANTS[1]

S. T. Dexter, W. E. Tottingham and L. F. Graber

(with two figures)

The injury of plants occasioned by winter conditions has been a subject of great interest and importance to plant breeders and agriculturists. The development of hardy strains has been, and is, one of the principal means of combating losses caused by injury from cold, although cultural methods, as well, have been adapted to help the plant survive the rigors of its climatic environment.

Recognition of the degree of hardiness has necessitated field trials commonly requiring extended periods of time, during which weather conditions may be too mild to test the relative hardiness in one season, while so severe in the next that hardy as well as tender varieties and strains are severely injured. It is quite evident that a rapid means of measuring relative degrees of resistance to cold of strains and varieties of plants, grown with various cultural procedures, would hasten progress in the work of plant breeders, as well as facilitate investigations of the physical and chemical nature of the hardening process, and its relation to the resistance of plants to low temperatures and other climatic conditions.

No attempt will be made to review the literature on hardiness or reactions of plants to freezing in reference to changes in permeability, colloidal structure or compositional variations. It seems to be generally accepted that the injury, or killing of tissue, by cold, or by any other means, involves the disorganization of the substances essential for carrying on the processes of life. With such disorganization, it is well recognized that the cell loses its capacity to regulate the diffusion of its soluble contents. Upon this basis, it was assumed that the degree of injury from low temperature, to overwintering and other plant structures might be correlated with the exosmosis of electrolytes and other materials following exposure to cold. Such outward diffusion of electrolytes can readily be estimated by conductivity measurements. Other investigators have applied conductivity methods to problems involving the viability of seeds, and the injury of plant and animal tissues.

To test this hypothesis, alfalfa roots of three varieties of known hardiness (Grimm, Utah Common, and Hairy Peruvian) were frozen under con-

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trolled conditions, for various lengths of time, and at various stages of growth throughout the autumnal season. The plants used were grown under fertile soil conditions from seed sown on July 13, 1928, in plats on the University Farm at Madison, Wisconsin. They were not cut that season, and the stands in all plats were excellent. They survived the very favorable winter season of 1928–29 without apparent injury.

During the growing season of 1929, a half of each plat was cut twice, on July 9 and September 12, when the plants were in full bloom, while the remaining half of each plat was cut four times, on June 22, July 9, August 13, and September 12. This latter treatment was one which would greatly inhibit the storage of organic foods in the roots, while the two-cutting treatment would provide for an abundance of such reserves.

Preliminary work during the early autumn showed that much more extensive outward diffusion into distilled water occurred when the roots had been frozen than when they were uninjured, but no varietal differences were observed. On September 21, 1929, a standardized procedure was first applied to this study. Roots were dug from the plats of alfalfa cut only twice and such alfalfa was therefore "high" in reserve food content. They were trimmed at the crown, to leave buds for further development, cut to a uniform length of seven inches, then washed hurriedly but thoroughly in running water, by taking a small bunch of roots and rubbing them between the hands, thus giving little opportunity for exosmosis in the washing process. The roots in bunches of several hundred were then covered with a damp cheese cloth, whereupon gradual evaporation brought the specimens to a uniform condition of surface moisture.

Samples consisting of 8 roots, trimmed at the base when necessary, to weigh 20 grams, were selected and placed in Pyrex test tubes (1 x 8 in.), closed with rubber stoppers. Five such tubes of each variety were placed in a circular rack, for each freezing treatment. The freezing was carried on in a cylindrical ice cream can of five gallon capacity, in a large thermostatically regulated electrical refrigerator, where temperatures ranged between $-8°$ C. and $-9°$ C. The roots were left at this temperature for 1.5 hours, 4 hours, and 15 hours. In each case, five tubes of roots of each variety were similarly prepared, but not frozen. Thus, 15 tubes were not frozen but were used as checks; 15 were frozen for 1.5 hours; 15 for four hours; and 15 for 15 hours. Each tube contained 8 roots, weighing 20 grams. After freezing, the roots remained in the tubes at room temperature for several hours. Three tubes of each variety from each freezing treatment were selected for transplanting in the greenhouse, where their recovery and subsequent growth might be observed. The two remaining tubes of each variety, from each freezing treatment, were placed in a rack.

This rack was suspended in a thermostatic water-bath, at 25° C., and to each tube, in succession at 2.5 minute intervals, 50 cc. of distilled water was added from a pipette. This water completely covered the roots. The tubes of roots remained in the bath for a ten-hour period.

At the expiration of this time, the liquid about the roots was withdrawn from each tube in succession, with a pipette, redischarged into the tube for mixing, and then again withdrawn and transferred to a conductivity cell, where the reading of resistance was taken to the nearest ohm. This precaution of thorough mixing appeared, upon investigation, to be unnecessary, but was nevertheless adhered to. The roots were removed from the tube and the solution was preserved for further chemical study. In this way, each tube of roots had an equal time for exosmosis into water. In several preliminary experiments it had been found that there was little change in conductivity after ten hours, and this interval was, therefore, chosen for all later experiments.

The freezing periods of 1.5 hours, 4 hours and 15 hours were chosen after some study, in the hope that the brief freezing would show slight injury, while the fifteen-hour freeze would completely kill all varieties. The one and one-half hour freeze was discontinued after four trials because the injury to the roots, as shown by the greenhouse checks, was slight in the later part of autumn. The fifteen-hour freeze was sometimes omitted to permit other experiments to be run. The four-hour freeze was run regularly at weekly intervals.

The average values for the specific conductivity, determined in duplicate in each case, and expressed in reciprocal ohms, on each date, for each variety are given in table I, and are represented graphically in figure 1. The figures presented are for the four-hour freeze.

TABLE I

SPECIFIC CONDUCTIVITIES ($\times 10^6$) IN RECIPROCAL OHMS, OF WATER EXTRACTS FROM ROOTS OF ALFALFA, AFTER FREEZING FOR FOUR HOURS. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS

VARIETY	SEPT. 21	OCT. 11	OCT. 18	OCT. 25	NOV. 1	NOV. 8	NOV. 15	NOV. 27
Grimm	1632	1250	1015	921	549	781	629	484
Utah Common	1657	1255	1235	1200	1177	1215	1270	879
Hairy Peruvian	1624	1459	1375	1535	1365	1447	1257	1475

No varietal difference was found in hardiness as measured by exosmosis from samples dug and frozen September 21, but during the latter part of October and the month of November prominent varietal differences are evi-

dent. This indicates that a very definite hardening process occurs in Grimm alfalfa during the fall period, while Peruvian has about the same resistance to cold on November 27 as on September 21. The Utah Common is intermediate throughout this period, but the hardening process seems to be delayed until the advent of freezing weather on or about November 19.

Apparently, the reaction of the plant to the climatic environment of the autumn period has much to do with its resistance to cold. The external appearance of these varieties growing under field conditions was markedly different during October and November. The last cutting was made on September 12, but owing to dry weather, there was very little growth recovery in any variety until September 28, when regeneration was stimulated by abundant rainfall. The character of this recovery varied widely in the different varieties. The growth of the Peruvian was rapid and up-

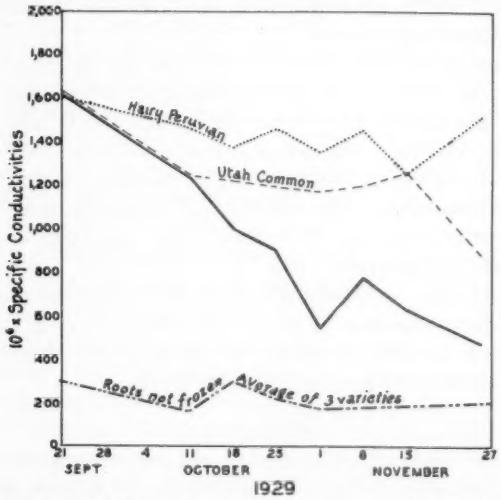

FIG. 1. Specific conductivity of water extracts from roots of alfalfa after freezing for four hours, and with an interval for exosmosis of ten hours. (Solid curve represents Grimm).

right, and on November 27 the frozen top growth measured about 14 inches. The Grimm alfalfa grew very slowly, and instead of being upright, was semi-decumbent. Just prior to freezing weather, the stems averaged about three or four inches in length. The Utah Common alfalfa was somewhat intermediate between the Grimm and the Peruvian with respect to its top growth. This description of the autumnal development of the three varieties is but another way of expressing the differences in dormancy.

No killing frost occurred until late in the season, and the top growth of the Peruvian was not killed until November 19, while the ground remained unfrozen until November 21. On November 27, when the last samples were dug for the conductivity experiments, the ground was frozen to a depth of about five inches. The exosmosis of all such samples tested without further freezing showed no injury from this frozen condition of the soil, while additional artificial freezing for four and fifteen hours (figures 1 and 2) caused considerable damage to the roots. The outward

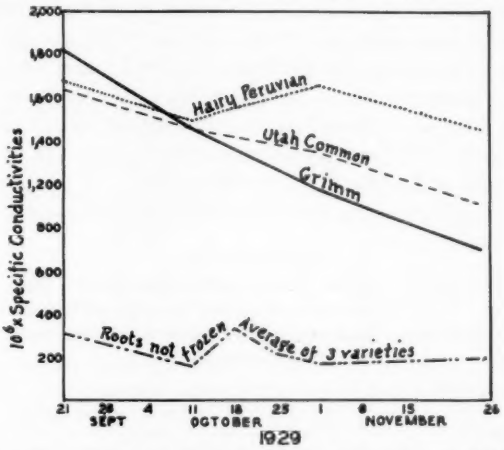

FIG. 2. Specific conductivity of water extracts from roots of alfalfa after freezing for fifteen hours and with an interval for exosmosis of ten hours.

diffusion of electrolytes is also indicated in table II by conductivity measurements after a fifteen-hour freezing treatment. Each value is the average of two determinations. This table is expressed graphically in figure 2. Again it is evident that Grimm alfalfa was no more resistant to cold on September 21 than were the other varieties, but in November, prominent differences are evident.

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$) IN RECIPROCAL OHMS OF WATER EXTRACTS FROM ROOTS OF ALFALFA FROZEN FIFTEEN HOURS. AN INTERNAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS

VARIETY	SEPT. 21	OCT. 11	NOV. 1	NOV. 27
Grimm	1820	1457	1191	696
Utah Common	1647	1463	1357	902
Hairy Peruvian	1680	1499	1689	1572

In order to check the method against another well established variation in hardiness, an experiment was carried out on November 8 and November 15 with alfalfa plants dug from the half of each plat of alfalfa cut four times, so as to facilitate a comparison with the plants cut only twice. With each variety, and in every case, without exception, the roots of alfalfa with low organic food reserves, due to four cuttings, gave a higher value for the specific conductivity than the corresponding roots with high reserves, from plants cut only twice. It has been shown that frequent cuttings lessen the hardiness of alfalfa and such results are corroborated by the conductivity measurements. Table III gives the averages of the two pairs of determinations, which checked very closely, and which were made on roots dug on November 8 and November 15. Differences between Grimm alfalfa with high and low reserves are pronounced, as are also the differences in the Hairy Peruvian with reference to reserves. The Utah Common did not show such wide differences in this respect. In accordance with the results of other investigators, the roots of the several varieties as sampled

TABLE III

AVERAGE SPECIFIC CONDUCTIVITIES OF THE EXTRACTS FROM ALFALFA ROOTS HIGH AND LOW IN RESERVE FOODS. PLANTS WERE DUG ON NOVEMBER 8 AND 15, AND THE ROOTS WERE GIVEN A FOUR-HOUR FREEZING TREATMENT. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS

VARIETY	CUTTING TREATMENTS	
	TWO CUTTINGS	FOUR CUTTINGS
Grimm	705	1145
Utah Common	1243	1346
Hairy Peruvian	1302	2093

after the four-cutting treatment showed a significantly lower percentage of dry matter than those receiving the two cutting treatment. In percentage dry matter as well as in conductivity, the Utah Common, however, showed distinctly less difference between the roots with high and low reserves than the other varieties.

As another means of distinguishing differences in the concentration of the exudates in the exosmosis test, the dipping refractometer was used, and was found very useful in detecting the larger variations. The instrument is not as sensitive as the conductivity apparatus and the slight turbidity of the solution often made the readings uncertain when small differences might have existed. No attempts were made to clear the solutions before examining them. The turbidities of these solutions, however, gave evidence of the degree to which exosmosis had proceeded, and very commonly the

greater turbidity of the solution from the Peruvian distinguished it from the Utah Common, which in turn was more turbid than that of the Grimm variety. Preliminary experiments indicate that the percentage of solids in the solutions as determined by simple evaporation may be definitely correlated with the conductivity figures. Hydrogen-ion concentration, determined on only one of the experimental dates, October 18, gave no definite correlation with exosmosis and freezing injury. The volume of the precipitate with lead acetate appeared, superficially to be about the same for all varieties.

The concentration of sugars in the solution was also followed throughout most of the season, but no fixed relationship appeared to hold. In general, the Utah Common showed greater exosmosis of sugars than the others, and Grimm showed the least.

In order to correlate the amount of injury from each freezing treatment with subsequent growth, twenty-four plants of each variety were transplanted in soil under greenhouse conditions. In general, the development of these plants gave definite indications of a distinct correlation between high conductivity and freezing injury. It is difficult, however, to express the degree of growth response in specific terms. While the growth recovery after freezing was greatest with the Grimm and always the least with the Peruvian, there were wide variations in different plants of the same variety. Thus, some roots were severely injured at the lower end, but the crown survived sufficiently to send out shoots, while others were injured at the crown and entirely failed to give growth, although the bulk of the root appeared to be relatively sound. Still other roots failed to regenerate even though they seemed to be very slightly injured. The renewal of growth by the plants subjected to the freezing treatments is often very slow, especially when they have been severely injured. In some cases plants were rejected as dead after a period of several weeks, whereas growth finally began after further opportunity. Roots usually die, however, if growth does not occur within three or four weeks, and late growth is always sparse. It is, therefore, difficult at this time to present concise data on the regeneration of growth in the greenhouse, after the freezing treatment. In every case the Peruvian, frozen one and one-half hours, was markedly slower in its early growth recovery than the Utah Common and the Grimm. In no case, did plants other than Grimm survive the four-hour freeze. It seems likely that the maturity of the buds at the crown may be an important factor in regeneration after freezing.

As a further aid in the determination of relative hardiness by the principle of exosmosis from injured tissue, the following colorimetric tests have been tentatively tried on some series, with very promising results. It

must be emphasized that the values of determinations of a given ion need not necessarily be in the same ratio as the conductivity values. The organic materials as well may interfere with the tests. However, in this experiment, tests for the chloride ion appeared to correlate very well with the conductivity measurements, as far as they have been carried out. Such tests may well supplant the conductivity measurement in some cases, and in others, they may supplement it.

To compare the concentrations of the chloride ion in the water extract of the roots after freezing, 10 cc. of each solution was treated with 1 cc. of 5 per cent. potassium chromate solution and 15 drops of N/100 silver nitrate. In the absence of chlorides, the solution turns a deep red, or in other terms, presence of chlorides in the extract inhibits the development of the red color of silver chromate. If there is sufficient chloride to precipitate all of the silver, the original yellow color will remain. Solutions from roots of Hairy Peruvian alfalfa, frozen 4 or 15 hours, show much less coloration of red silver chromate than solutions from roots of Utah Common, and in turn, Utah Common show less than Grimm. Thus, Peruvian has given more chloride to the solution, by exosmosis, than the other varieties. The test when applied to the extract from unfrozen roots shows practically negligible exudation of chlorides, and the solution turns a deep red on the addition of the silver nitrate. The colors produced in the solutions remain unchanged for several hours and may be referred to a series of standard chloride solutions, similarly treated for quantitative comparison from week to week.

Organic substances interfered with the tests for nitrates with ferrous sulphate and sulphuric acid, but the colorations produced in 50 per cent. sulphuric acid appeared to correlate fairly well with the conductivity measurements.

These methods should be further standardized. Tests made for the calcium and sulphate ions were not sufficiently definite to appear of value and will not be described here.

A considerable number of possible variables have been investigated in this preliminary work. The size of the roots appears to make no difference in either cold injury or exosmosis, within the limits of our field conditions. Small roots may be cut longer than usual, or thick roots may be cut to one-third of ordinary length to make up the twenty grams of tissue. It does not appear to be necessary to use a given number of roots, so long as a given weight is employed. The roots may be cut off below the crown without affecting the experiment appreciably, but if the crown tissue is cut away, such roots are not suitable for greenhouse tests of growth recovery. Total ash determinations on the roots throughout the fall do not show

marked differences in the three varieties. The influence of soil added to the solution in considerable quantities was determined, and does not appear to be one of the larger variables. The rate of thawing may have considerable influence on the amount of injury and the amount of diffusion, but the influence of this variable was not measured. The rate of thawing, however, was quite uniform in all the trials reported in this paper.

The common precautions in conductivity work were employed. The tubes for exosmosis and their stoppers were soaked in distilled water for a day or two before use, and contamination by electrolytes was carefully avoided. With ordinarily careful technique, it does not appear that the unavoidable errors in the conduct of the measurements described are sufficient to affect, in a large degree, the accuracy of these determinations.

There are many incompleted problems left for further study, which will occur to the reader. The possible applications of the method to plants other than alfalfa is obvious. The writers plan to investigate some of these during the coming months, and to further establish the relationships that other variables may have in the reliability of the methods as outlined. It seems very likely that modifications, particularly of the colorimetric tests will be desirable, at least, in applying them to other plants.

Summary

A series of experiments and data has been presented indicating that the degree of resistance of plants to injury by cold weather may be measured by the diffusion of electrolytes and other substances from chilled or frozen tissues into water after such tissues have thawed. The amount of diffusion has been determined with alfalfa roots by conductivity measurements, which have been supplemented by colorimetric tests for chlorides. Within the limits of this investigation there exist correlations between known hardiness of alfalfa roots and the degree of retention of electrolytes by the tissue after freezing.

The writers wish to express their indebtedness to the Department of Physical Chemistry of the University of Wisconsin for the use of conductivity apparatus, and to the Dairy Department for the use of freezing equipment.

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DORMANCY AND GROWTH STUDIES OF THE AMERICAN LOTUS, *NELUMBO LUTEA*

WILLIAM C. MEYER

(WITH EIGHT FIGURES)

Attempts to establish the beautiful native American lotus in new locations have often been marked by failure, a fact which evokes scientific interest in the growth and dormancy habits of this plant. In spite of the fact that embryos have been known to sprout after two hundred years of dormany (**6**), it is doubtful if the plant reproduces from seed at all frequently in nature. The facile multiplication of tubers and the rapid growth of shoots from these create a competition for light and space which seedlings, if started under natural conditions, could probably not overcome. With the lotus in nature primarily dependent upon tubers for perpetuation, it is not surprising that florists in commercial production also rely chiefly upon them rather than upon seeds. The tubers produce a large flowering plant in a single season while the seedlings require at least two and sometimes probably as many as four years for floral development.

No record has been found in the literature of successfully growing the plant from seed. It is stated (**3**) that if holes are made in the fruits and they are then placed in a pond, their embryos are so delicate that they do not survive. In another case no signs of plants were found a few years subsequent to the planting of thousands of the fruits sown just as they came from the pods (**3**). The writer is personally acquainted with men who have had similar failures.

Scientifically, interest has centered largely in the germination of Nelumbo seeds. Old Indian lotus fruits obtained by OHGA from a prehistoric peat bed in South Manchuria are the most striking examples of protracted dormancy known to science (**6**). These were estimated to be three hundred to four hundred years old and certainly not less than two hundred years. A long period of dormancy has not been recorded for the American species but the fruit structure of the two species is practically alike and no sign of swelling occurs in either after being kept in water for several months (**2**).

OHGA (**6**) found concentrated sulphuric acid treatment to be the easiest method of overcoming this dormancy. Fifty per cent. chromic acid and concentrated potassium hydrate also overcome the impermeability after a longer period of time. According to his work, practically all of the fruit coat shows the cellulose reaction. SHAW (**9**) describes the cells as having a middle lamella of a mixture of pectic compounds and lignin, and a cellu-

lose lamella on either side which becomes very thick in the palisade cells composing the impermeable layer. She also describes a deposit of suberin in a lamella between the cellulose and the middle lamella of the palisade. In the cells bordering the stomatal cavities the suberin would be the material exposed to reagents. In her conclusions she states that "the only possible way to cause water to enter these stomatal cavities in *Nelumbo lutea* is to remove the fatty suberin lamella by some solvent. Aside from this method the only way to germinate these fruits is by filing the coats through the palisade layer, or by removing this layer by strong reagents."

Both OHGA and SHAW considered the stomatal cavities as places of attack but OHGA also considered the vascular scar at the basal end, the protuberance and the stylar canal, as points of water entry. After a thorough microscopical investigation he suggested a possibility of entrance at the stylar canal but said it would be rather difficult, and thought that it would be impossible at the other two points. In the present work, treatment with Schweitzer's reagent for seventy-five to two hundred hours made the fruits permeable, and the embryos developed normally. A three-hundred hour treatment destroyed the embryos. The reagent apparently gained entrance at the stylar end, that being the first portion of the coat to show swelling after the fruits were placed in water. SHAW probably is correct in stating that a fat solvent is necessary for dissolving the retarding layer in the stomatal cavities, but the statement that the only possible way to cause water to enter is by means of such a fat solvent, filing, or strong reagents does not appear to be correct, unless Schweitzer's reagent be classed as one of the latter. Further, in view of the specificity of Schweitzer's reagent, cellulose tissue, doubtless, participates in maintaining the impermeability of the fruit coats to water. Thus, a cellulose layer of cells may be chiefly responsible for the protracted dormancy of Nelumbo fruits.

Despite the searching investigations of OHGA (**4, 5, 6, 7**), SHAW (**9**), JONES (**2**) and others on the structure and behavior of the fruits, little is known concerning the development of seedlings or methods of establishing the plant in new locations by means of fruits or tubers. Difficulty in establishing new lotus beds suggests that the normal development of the plant may be profoundly influenced by certain specific environmental factors. This report deals primarily with the effects of certain factors on the growth of *Nelumbo lutea*.

Methods

The physico-chemical nature of the soil, as well as temperature, were thought to have some influence on the development and distribution of the plant. The northern limit of distribution is at Lake Pepin in the Mississippi River between Minnesota and Wisconsin. The low temperature of northern streams and lakes may prohibit further advance northward.

Higher temperatures from 20° to 30° C. have been found to greatly accelerate growth, while below 15° C. growth is very limited. This was partly determined by growing both seedling and large-tuber plants at 10°, 15°, 18° and 25° C. The accompanying photograph of seedlings (fig. 1) clearly

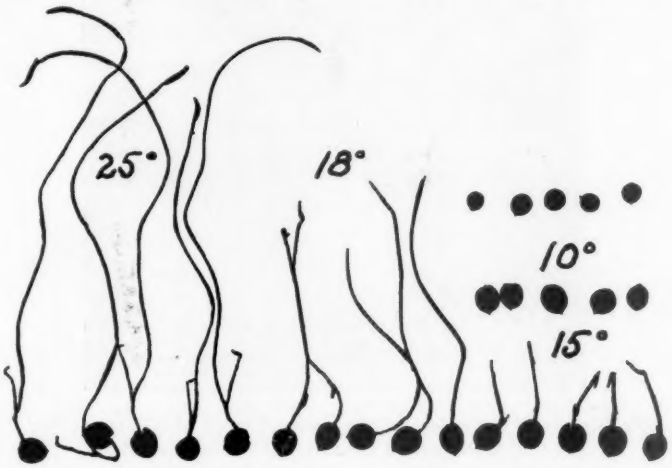

FIG. 1. Growth of seedlings in tap water at different temperatures. Fruits at upper right not treated to break impermeability.

shows that growth is directly related to temperature. The higher the temperature the more rapid the growth for the temperatures used. The upper thermal limit at which the plant will grow was not determined.

Slow growth, due to low temperature, would not permit successful competition with vigorous northern plants; but this does not explain the inability to establish the plant in new locations of similar temperature. The northward advance may also be prevented by the depth of winter frost. That the winter-tubers are destroyed by freezing was readily determined by freezing the tubers in water, at $-2.0°$ to $-2.6°$ C., for two hours. BISSET (**1**) and TRICKER (**10**), authorities on water gardening, both state that the tubers are destroyed by freezing. Destruction of the tubers in this manner may account for the lack of success in a new location of shallow water. But the depth of ice rarely exceeds two feet even in our northern lakes. As the tubers are located six to twenty inches in the soil beneath the water it appears that other factors are involved in nature.

Hydrion concentration has been found to be very influential in the development and distribution of plants (**11, 12, 13**), but little is known of its effect on the germination and development of aquatic spermatophytes. The large American lotus proved excellent material for such observations.

For a study of the effect of various hydrion concentrations galvanized iron tanks, 60 x 60 x 120 cm., painted within with an impermeable asphaltum paint, were used (fig. 2). Additions of sulphuric acid and sodium

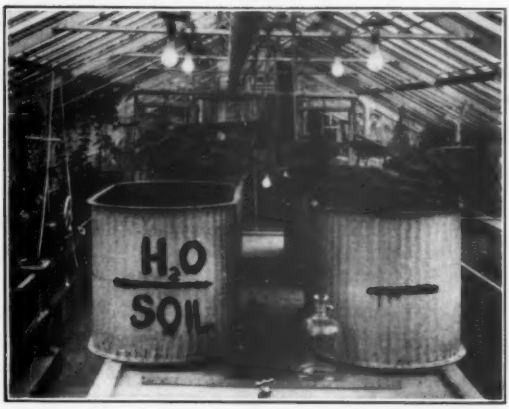

FIG. 2. Photograph of equipment for the study of effect of pH on seedling development. Connections for aëration and lighting are shown.

hydroxide gave the desired pH values. The continued shifting toward the neutral point by both acid and alkali solutions was corrected by regular additions of small amounts of normal solutions. Daily colorimetric tests were made and checked potentiometrically at intervals.

After the experiment had been running a month and a half, air was bubbled into the water through blocks of tulip poplar to maintain circulation and to prevent the growth of surface organisms. Electric light was used in addition to the daylight during the winter months (fig. 2), a one-hundred watt bulb being placed above each tank. The lights were turned off during the normal period of darkness. Plants were also grown in a cement basin (painted inside with the asphaltum paint to avoid rise in alkalinity). A small stream of tap water was allowed to run continually through the tank, resulting in a pH of 7.7.

Data and discussion

The plants grown in the basin showed a normal development as evidenced by their similarity to plants grown under natural conditions the previous year in a preliminary study. Four leaves form and then the rhizome. There is no hypocotyl and roots do not appear below the cotyledons but at the bases of the true leaves (fig. 3). The second internode of the rhizome becomes enlarged into a tuber (figs. 3 and 4) and the plant

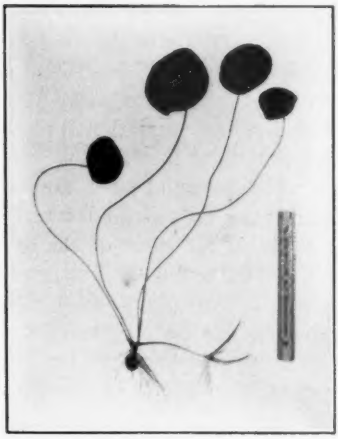

FIG. 3. Photograph of typical seedling plant at age of two weeks showing the four peltate leaves which were present in embryonic form within the one-seeded fruit (remains of which are still attached), roots from the bases of these leaves and first node of rhizome, and the tuber developed in the second internode of the rhizome.

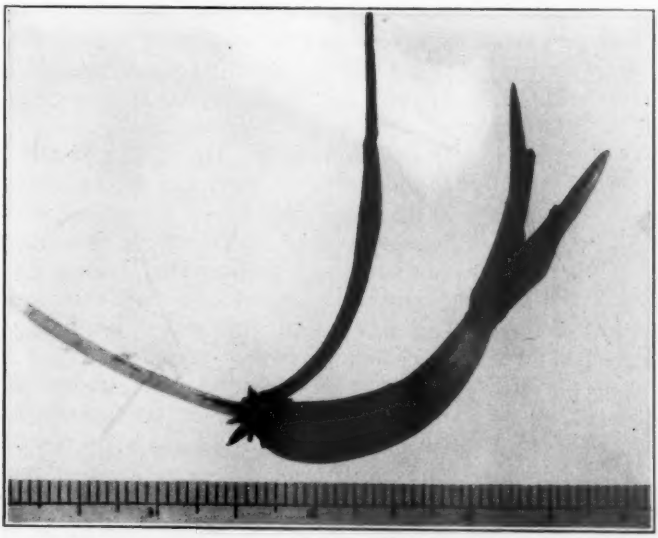

FIG. 4. Seedling tuber, two weeks after germination of the seed at 26° C. Growing point at the extreme right, the other two points being young leaves within stipular sheaths.

remains dormant for about three weeks. The first four leaves then begin to decay and if the underground parts were not examined, one would be apt to think the plants were dying. This striking behavior is a perfectly normal condition. Within three weeks after the temporary arrest in development, new tissue develops from the tuber and its enlargement is very rapid, the rhizome extending through the soil, and leaves and roots appearing at the nodes which occur at quite regular intervals. Branches of the rhizome also occur at the axils of the leaves (fig. 5).

For a preliminary study of the effect of the hydrogen ion, pH values were established at 11.5, 9.0, 6.0 and 3.3. The two extremes were replaced by plantings at 9.7 and 4.5. The plants were most easily grown at pH 9.0, the one planting being successfully carried for the seven months of the experiment. Temperatures taken at the soil surface varied from 19.5° C. in the winter months to 32° C. in the summer, the average from March 22 to July 22 being 25.4° C. The third and later leaves unrolled the laminae on the surface and during the last month of the experiment projected themselves a considerable distance above the water. The characteristic growth habit of the plant is shown in fig. 6.

Comparison of plants at pH 9.0 and pH 4.5

A comparison of the pH 9.0 plants with those grown at pH 4.5 again shows that growth at the lower pH was much more rapid. When the former were six months of age and the latter three and one-half months, the average area of laminae at pH 4.5 was 31.9 sq. cm. greater than that of laminae of pH 9.0 plants. During the following month this difference increased, the area of acid laminae exceeding that of the alkaline by 100 sq. cm. The height of the leaves above the water and the diameter of their petioles were also greater in the acid a month later. No tubers were formed at pH 4.5. Starch is ordinarily the chief food reserve in these tubers and it appears that acidity interfered with carbohydrate storage and tuber formation. The laminae, upon microscopical examination showed looser arrangement of tissue in the acid plants. This was evidently due to the rapid growth in the acid. The slower growth at pH 9.0 was at first thought to be due to a greater osmotic pressure in that solution, making absorption of materials from the water and soil more difficult, but determinations of freezing points (**8**) of the solutions in the pH 4.5 and pH 9 tanks showed a low osmotic pressure (fig. 7). Growth differences are thus the direct result of variations in the hydrion concentration.

The seedlings at pH 4.5 began to develop brown spots in the laminae about a month after their planting. Yellowish wilted spots appeared in the spaces between the larger veins four to five days after the laminae

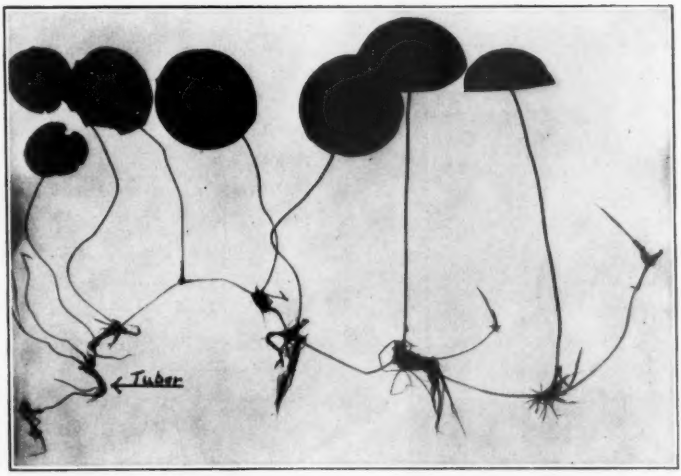

FIG. 5. Seedling plant at age of seven weeks, at 26° C. Roots from the bases of the first four leaves at the extreme left, the tuber in the second internode and the growing point of the main rhizome at the extreme right.

unrolled, then turned brown as the cells collapsed and the tissue became dry. The dead spots spread over a larger area and soon coalesced, killing

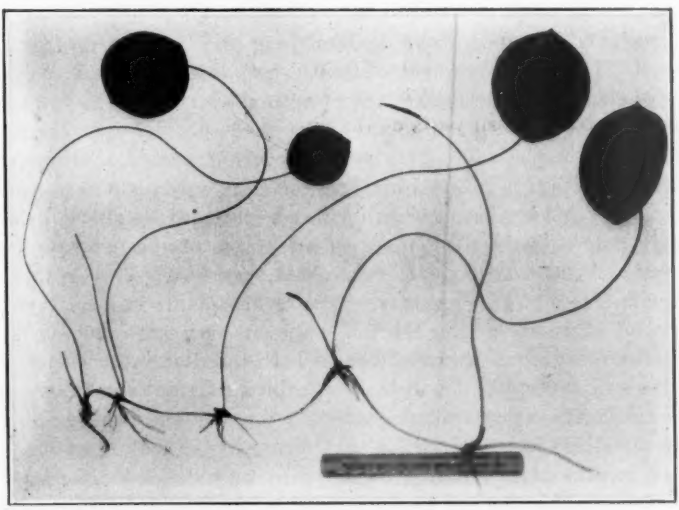

FIG. 6. A typical plant at pH 9.0, four months from seed.

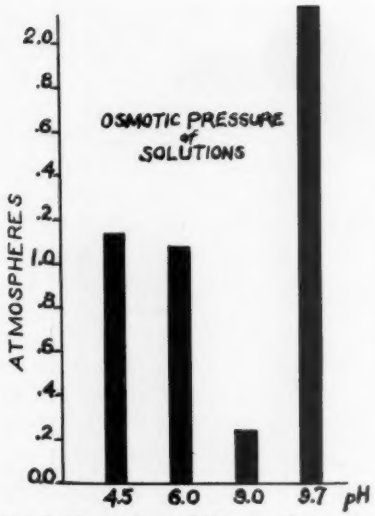

FIG. 7. Comparison of the osmotic pressures of solutions in which the plants were grown.

the laminae. Tying leaves up above the water was found to be a remedy for the trouble and from then on, nearly all were given support until the petioles of the more recent leaves raised them above the water without assistance. Sections made through spots showed no parasitic organisms. The collapse occurred first in the layer of tissue beneath the palisade. After the leaves commenced to rise above the water, unaided, there was little trouble in caring for the plants.

In the present work the plants were found to grow in a pH range from 4.5 to 9.7. Acidity, if not in excess, favors growth but the plants also develop normally at pH 9.0. Tests made on the soil and water of several natural lotus beds show a hydrion range from pH 5.2 to 8.7.

Soil type effects

The plants described above as developing at pH 7.7 were grown in a loam soil. This basin, however, contained both loam and sand, each covering one-half of the floor. The water continually running in from a tap, circulated freely to all parts of the tank. The only difference in the two portions was in the soil. This arrangement permitted a study of the influence of soil types. Equal numbers of fruits were planted in loam and in sand after having been treated with concentrated sulphuric acid. At an early date a distinct difference in the plants of the two soils became apparent. A month after planting it could very clearly be seen that there were more leaves of greater surface area on the plants in loam than those in the sand. Two weeks later this difference was even more striking (fig. 8). Tubers were found on the seedlings of both soils but were larger in the loam than in the sand. The plants remained dormant for three to four weeks and again, upon resuming growth, those in the loam greatly surpassed the others in rapidity of development.

Five months after planting there was a very decided difference, definitely showing that the soil type is probably the most important factor in the development of Nelumbo. The foregoing conclusion is based upon the

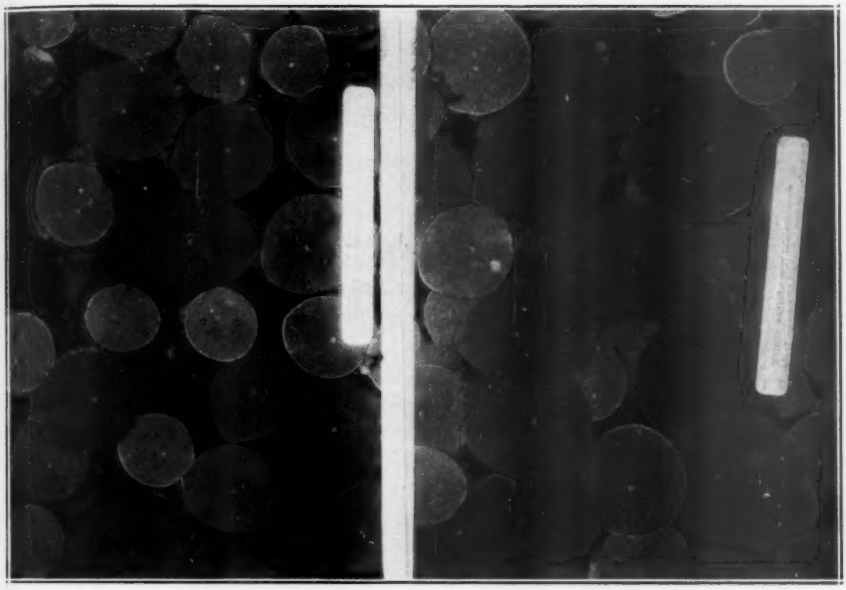

FIG. 8. Effect of sand and loam soils on growth of Nelumbo in the same pool at pH 7.7. Left, sand; right, loam. All plants six weeks old.

fact that plants in the large culture basin were bathed by the same circulating solution and hence by the same nutrient solutes. Despite this uniformity of the culture solution, the difference in the plants of the loam and sandy soils was so striking as to make inescapable the inference that the edaphic environment has a much greater effect than the aqueous environment on growth. Failure to fully appreciate this fact probably underlies many failures to establish the lotus in new localities. Whether this edaphic influence is primarily physical, nutritive or biotic remains a matter for later study. Organic soils, however, are preferable to mineral soils, especially those of siliceous type.

Conclusions

1. Despite the long delayed germination and impermeability of the fruit coats, Nelumbo plants can be grown successfully from seed.

2. The plants can tolerate a considerable hydrion range. Water-gardeners and lotus enthusiasts should have little trouble as far as acidity and alkalinity are concerned in growing the plant in pools ranging from pH 4.5 to pH 9.0.

3. Difficulty in establishing *Nelumbo lutea* in new locations is probably not ascribable to low temperatures or unfavorable hydrion concentration, but rather to unfavorable edaphic factors. Mineral soils, especially those of the siliceous type, are inferior to organic types for the development of the lotus.

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TENTATIVE CLASSIFICATION OF SYMPTOMATIC TYPES OF "TOMATO POCKETS"[1]

HAMILTON P. TRAUB, W. S. HOTCHKISS,[2] AND P. R. JOHNSON

(WITH FIVE FIGURES)

It is conservatively estimated by prominent tomato growers and shippers in Texas that annually more than 15 per cent. of the Texas tomato crop is lost due to a condition commonly known as "Tomato Pops," or "Tomato Puffs." The trouble is serious in all the Southern States. At present the cause or causes of this abnormal condition are unknown, presenting a challenge to the Experiment Station worker in the South. The present paper is presented in order to bring this fruitful field for research to the attention of workers generally.

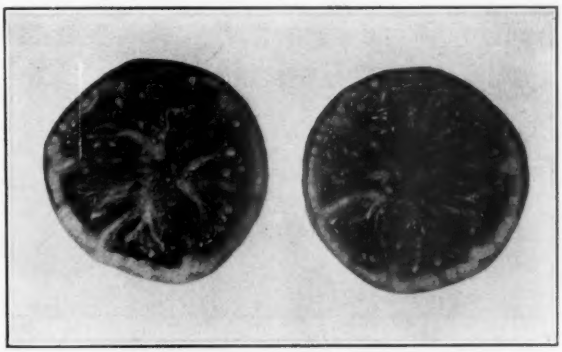

FIG. 1. Marglobe, fruit with orange-yellow exterior; symptomatic type A; marketable before edema-like symptoms develop.

Method of attack

During the season 1928, as a part of the general subject of quality in fruits, experiments were initiated at the Texas Station with the object of determining the cause or causes of this abnormal condition of tomato fruits. Although these experiments are in progress it is desirable that the problem receive attention also at other Stations since it concerns all the Southern States, California, Mexico and the Caribbean Isles. Greenhouse tomato growers in the North may also be interested. The method of attack includes a consideration of (a) the morphology and physiology of the dis-

[1] Published with the approval of the Director as Paper no. 97 of the Technical Series of the Texas Agricultural Experiment Station.

[2] Deceased, July 14, 1928.

eased fruit;[3] (b) the possible causal correlation between this abnormal condition and virus infection;[4] (c) the temperature, moisture and humidity factors, and (d) other factors, such as pollination, light, soil solution, etc., singly and in combination.

LESLEY and ROSA (**2**) state that one self-fertilized line was observed which produced a much larger proportion of soft and "puffy" fruits than the parent. Breeding for resistance should apparently be given consideration as a possible method of solving the problem.

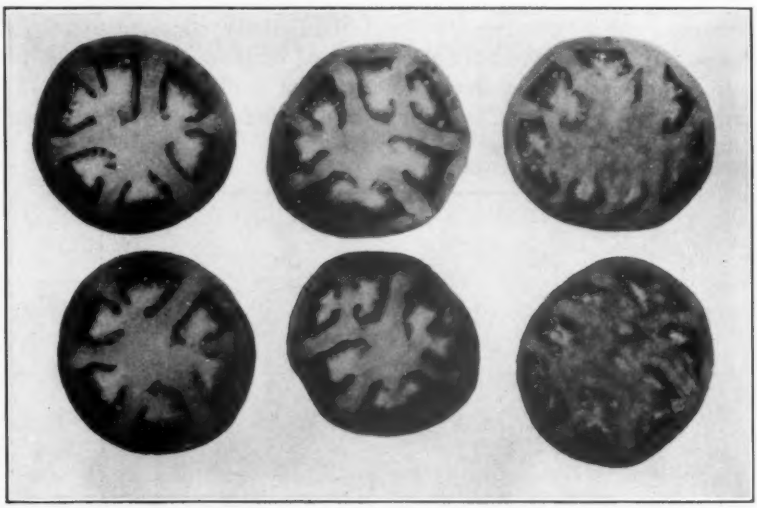

FIG. 2. Marglobe, green fruit; symptomatic type B; marketable.

Classification of types

The work as outlined is a cooperative undertaking in which many are taking part, and for this reason it was considered advisable to present a preliminary classification of the types of symptoms encountered, for the convenience of those engaged on separate phases of the research. In July, 1928, at Texas Substation no. 2, Troup, over 5,000 diseased fruits were examined. The apparently distinct symptomatic types of the disease observed are portrayed in figs. 1 to 5, inclusive. There is apparently a gradual gradation from one type to another, but for practical reasons four types have been named.

[3] Prof. F. S. JAMISON is engaged on this problem.

[4] Drs. J. J. TAUBENHAUS, and W. N. EZEKIEL, and Mr. W. H. FRIEND are cooperating on this phase of the problem.

Aside from the possible importance of severity of attack, the symptoms apparently depend upon the stage of development of the fruit when it becomes subject to the disease:

(a). When the fruits are quite mature at the time of becoming subject to this condition they are apparently characterized by senescence of vascular bundles in the fruit, showing as white areas. The development of the ectocarp apparently is retarded in development impeding the normal

FIG. 3. Marglobe; green fruit; symptomatic type C; not marketable.

formation of lycopin. As a result, the fruit is of an orange color on the outside. At an advanced stage in the development of the fruit, the placental tissue and sometimes the meso- and endo-carpal tissues in the interior of the fruit mature almost normally, and the fruit becomes "dead ripe" when the ecto-carpal tissue is still in a yellow condition. This results in a watery interior similar to edema or dropsy. See fig. 1, symptomatic type A. This type was observed in only a limited number of cases, and is marketable before edema-like symptoms develop.

(b). Fruits affected at an earlier stage of their development apparently show large areas of opaque, degenerated tissue in the center of the fruit conspicuous as white areas radiating outward in the locular walls and placentae. See fig. 2, symptomatic type B. This type is marketable.

(c). Fruits affected at a still earlier stage of their development are apparently characterized by marked contraction of tissues, especially pla-

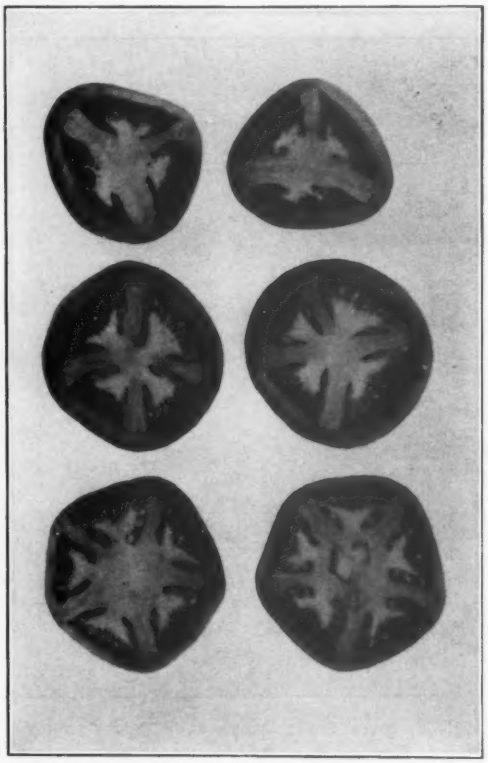

FIG. 4. Gulf State Market; green fruit; symptomatic type C; not marketable.

cental and locular-wall tissues. Both surfaces of the locular and outer pericarpal walls contract; the placental tissues contract toward the center leaving an "open space" or "pocket" and the outer pericarpal wall is tightly stretched from one locular-wall to the other. This leads to distinct angularity. The fruit is also relatively light in weight. This is the symptomatic type commonly recognized and is not marketable. See figs. 3 and 4, symptomatic type C.

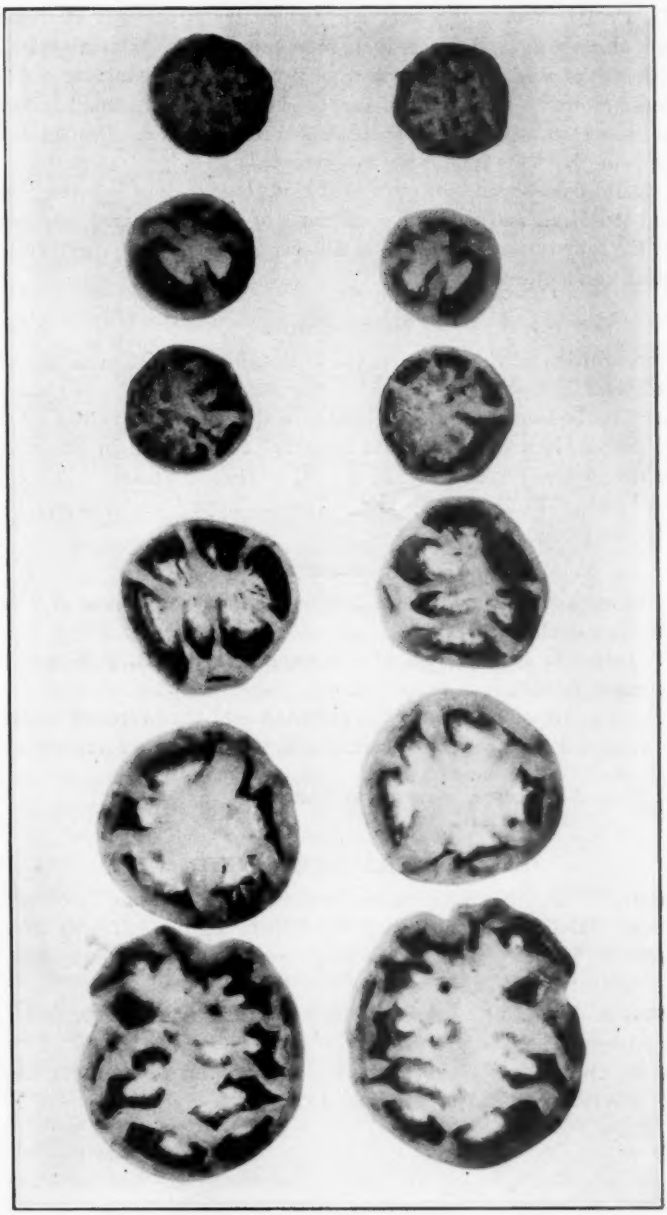

FIG. 5. Marglobe, green fruit; symptomatic type D; not marketable.

(d). Fruits affected at a very early stage are apparently characterized by total absence of seeds or reduction in number; in extreme cases, with total absence of seeds, the fruit is wholly vegetative resembling a dwarfed "witches broom." Most fruit of this type observed were small in size, but medium sized and even large specimens were observed. See fig. 5, symtomatic type D. This type is not marketable.

It should be realized, however, that this classification is merely suggestive and tentative, and that the occurrence of the last named, and possibly each of the four types may be due to different causes. Only further experimentation can settle these points.

Nomenclature

In recognition of the fact that the outstanding symptom of the typical diseased fruit, symptomatic type C, is the presence of an "open space" or "pocket" in the locules, and that there is no distention but rather a contraction of tissues, the name "Tomato Pockets" is proposed in preference to the less descriptive terms, "Puffiness" (**2**); "Hollow Tomato" (**1**); "Puffy Tomato" (**4**); "Puffing" (**3**) and "Tomato Pops" or "Tomato Puffs," (local Texas terms).

Summary

1. The subjects under investigation concerning the problem of "Tomato Pockets" are outlined.

2. A tentative classification of symptomatic types, A, B, C, and D, of the abnormal condition is presented.

3. Reasons are advanced for the acceptance of the proposed descriptive term "Tomato Pockets" in preference to less descriptive local terms.

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THE RÔLE OF MANGANESE IN THE NUTRITION OF LEMNA[1]

NORMAN ASHWELL CLARK AND CLAUDE L. FLY

(WITH THREE FIGURES)

The place of manganese in the nutrition of the green plant has been investigated in detail for some years, and numerous claims have been advanced with regard to its function, but there does not seem to be any considerable consensus of opinion as to its effect on the composition of the tissues or on the reproduction of the plant. There seems to be an equal uncertainty whether manganese should be classed as one of those elements which have been claimed as essential for growth (although in small quantities), or if it should be put with those which stimulate but are not absolutely necessary for the existence of the plant.

In 1920 OLARU published a thesis on the place of manganese in agriculture (**15**) and reviewed the work done throughout the world up to that time. In almost all cases manganese had proved favorable to the development of plants in soils, although large quantities were decidedly toxic. In liquid cultures the addition of suitable quantities of manganese stimulated both germination and development.

OLARU showed that this energetic action of manganese had been explained by the many different investigators, in almost as many different ways, including among others: catalysis of chemical action in the cell (BERTRAND); oxidation of toxic substances in the leaves (LOEW); production of chlorophyll (VAN DORN); rôle in photosynthesis (STOKLASA); stimulation of assimilation of elements (ROUSSET); chemical action on soil salts (MENOZZI); increased solubility of calcium salts and silicates (BERNARDINI); increased oxidation (SCHREINER); oxidation and stimulation of bacteria (SKINNER); permeability of protoplasm of cell to salts of calcium and magnesium (KELLY); ratio of iron to manganese (PUGLIESE).

By 1925 the amount of information had increased but without altering to any large extent the conclusions of OLARU. Influenced by the discussion on the essential nature of the vitamins in animal life, investigators gave more attention to the place of manganese as a possible essential element in plant life, in contrast to its stimulating action. BRENCHLEY (**5**) in her monograph on Inorganic Plant Poisons and Stimulants in 1927 sums up that manganese has a stimulating action on growth in small quantities, but large amounts are toxic to plants. The precise way in which this stimulation is accomplished is not known, and the "essential" nature of the element is as yet uncertain.

[1] Contribution from the Department of Chemistry, Iowa State College.

Several workers have recently reported experiments on plants for which manganese seemed an essential. HAAS and REED (**12**) found that with young orange trees they could not get good growth in culture solutions commonly considered "complete," when the young trees were grown for long periods in these solutions. Manganese was among the elements which it was necessary to add in traces to remove this condition. BISHOP (**4**) in Australia noted the presence of manganese in 25 species of Eucalypts. In these and in wheat, barley, maize, vegetables and fruits, the manganese was concentrated in the parts of the plants where there was the greatest chemical change. In his sand cultures he found approximately 5 parts per million was the optimum concentration in the nutrient solution supplied, but that neutralizing the acid solution with $CaCO_3$ enabled plants to grow in 50 ppm.

MCHARGUE (**14**) in 1927 reported the occurrence of a number of elements, including manganese, in Kentucky blue grass. From earlier experiments he had concluded that manganese is essential for the growth of plants, and is connected with chlorophyll or protein formation, or possibly with the stimulation of enzymes which split fats, sugars and starches, and render them available for young seedlings. He suggests here that it is the manganese which causes the deep blue color of the blue grass, and he proposes a theory connecting that element with vitamin production in plants. As most of the copper, manganese and zinc occurs in the germ of the seed, it may be connected with those vitamins which are found in the germ of the seed. Similarly the mineral elements supply the vitamin-forming material necessary for the growth of plants, and may become upon resynthesis the vitamin factors necessary for animal growth.

A number of these points can be studied very well in nutrient solutions. ASO (**3**) in 1902 used water cultures and stated that plants could develop normally in the absence of any trace of manganese, while WEIS (**17**) more recently states that manganese could not prevent chlorosis and that it did not influence the plants in solution. These conclusions are not in accord with those of HAAS, BISHOP or MCHARGUE.

The value of water plants for nutrient studies has been generally recognized, but the fact that they are specially adapted to the investigation of manganese has not been made use of to any extent. GÖSSL (**11**) showed that marsh and water plants generally gather up considerably more manganese than land plants; *Lemna trisulca*, for example, contained large amounts. In the experiments reported below, *Lemna major* (*Spirodela polyrhiza*) was used. As collected from ponds in central Iowa it showed a considerable amount of manganese in its composition.

Conditions under which the *Lemna* plants grow have been investigated in this laboratory for several years. In 1924 CLARK and ROLLER (**10**)

showed that the plants, if given a suitable medium of inorganic constituents, did not need the addition of organic matter in order to reproduce and to keep healthy, and that the "auximones" suggested by BOTTOMLEY were possibly of the nature of stimulants, but could not be classed as essentials for the growth of plants. Effects of intensity and duration of light on the reproduction were reported by CLARK in 1925 (**6**) and the influence of hydrogen ion concentration was investigated in 1926 (**7**). The medium developed in this way has been used by ASHBY and his coworkers (**1, 2**) in London with considerable success in the growth and reproduction of *Lemna minor*. A general review of some of the problems connected with vitamins and plant growth was given by CLARK in 1929 (**9**).

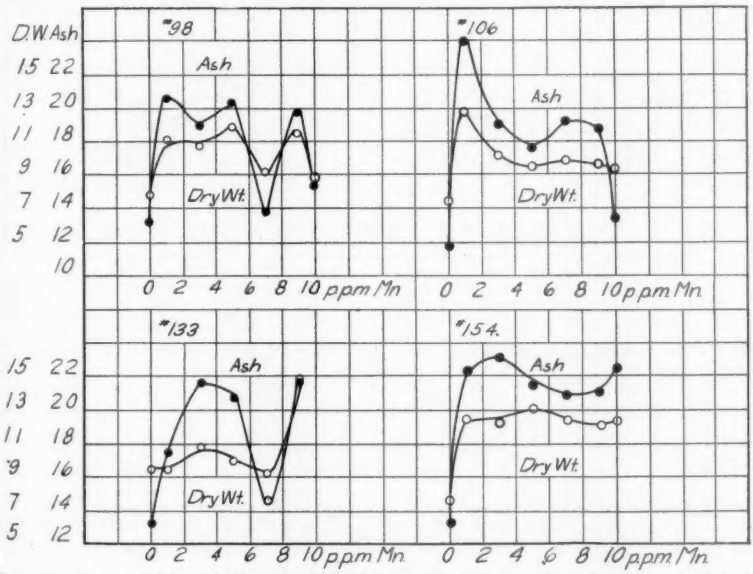

FIG. 1. Average dry weight and ash in plants with concentration of manganese 0 to 10 ppm. in the solution. Scale for ash weight is 10× dry weight.

The nutrient solution was used as indicated by CLARK (**6**) and the initial pH was adjusted when necessary to 4.8. Salts were purified by recrystallization as described by CLARK (**8**). The technique followed was altered slightly by substituting Erlenmeyer flasks for beakers, and these were closed with loose plugs of cotton wool. The flasks were placed in a thermostat in which the temperature was kept at 25° C. and exposed to sunshine; no artificial light was used. Controls were kept at all times in the standard solution along with the other flasks, and conclusions drawn from comparison

with these controls. As the light varied at different periods of the year, cultures grown at different times were compared only through the controls.

The stock plants were grown in the inorganic solution free of manganese for several months before being used for the experiments. They had thus passed through some 40 or 50 generations without access to manganese. The number of fronds was counted and the reproduction constant, K, calculated from $\log_{10}N - \log_{10}N_0 = K(t - t_0)$ (**6**). The fronds were dried and the average weight found. The ash and volatile matter were also determined. The method for obtaining the dry weight is somewhat different from that outlined by Su (**16**), who placed the plants for 30 minutes in a vacuum of 5 mm. at 50° C. In this laboratory the plants are dried in micro-cru-

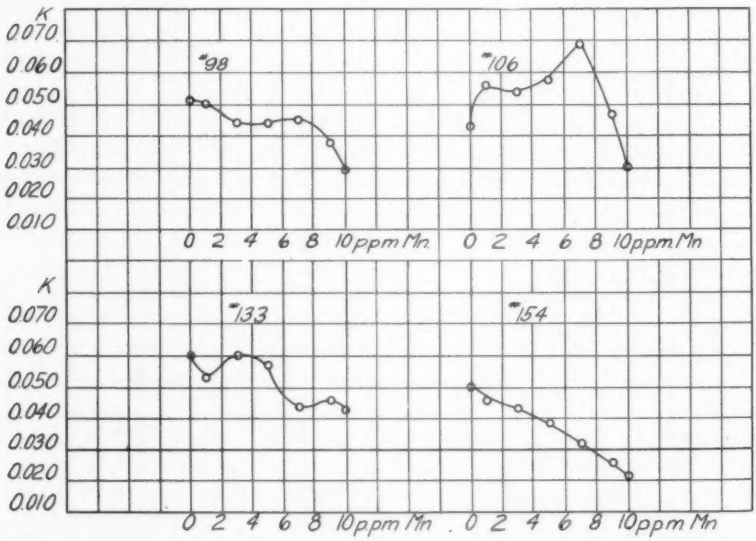

FIG. 2. Reproduction constant, K, for concentration of manganese from 0 to 10 ppm. in the solution.

cibles of platinum under a partial vacuum in a stream of dry air at 45° C. The platinum crucibles were placed in porcelain crucibles in the desiccating chamber, and when the plants were dry (half an hour or over night showed no change in weight) the lids were placed on the porcelain crucibles, and the whole transferred to a sulphuric acid desiccator until room temperature was reached; or the desiccating chamber was allowed to cool and the platinum crucibles transferred to a closed weighing bottle of known weight which was kept in the case of the Ainsworth microbalance (**13**). It was found necessary to have the weighing bottle in the balance. (If kept in the

drying chamber the condensation of moisture during the weighing is very variable.)

Figure 1 shows some typical curves representing the average dry weight and ash content of the fronds both in the medium without manganese and with additions of manganese chloride to make concentrations of that element from 1 to 10 parts per million. It will be seen that there is in every case a rapid rise in both dry weight and ash as the manganese is added until a maximum is reached at about 1 or 2 ppm., after which both are irregular to 10 ppm. Above 10 the manganese concentration becomes increasingly toxic and the plants die off rapidly in the higher concentrations. The rate

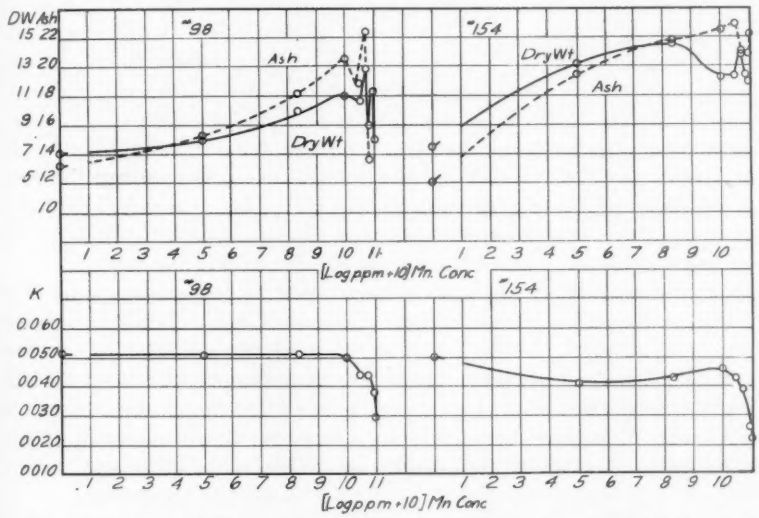

FIG. 3. Average weight and ash, and reproduction constant, K, with logarithm (plus 10) of the ppm. of manganese in the solution, as abscissa. Scale for ash weight is 10 × dry weight.

of reproduction, however, as represented by the constant, K, does not show any regular increase with the addition of manganese. This is shown in figure 2. From 1 to 10 parts per million there is a tendency for K to decrease, and in the region of these concentrations the plants frequently show loss of roots and brown spotting.

That about 1 ppm. of manganese is the concentration for maximum dry weight and maximum ash content under these conditions, is clearly shown in figure 3, where the abscissa is the logarithm of the ppm. When the average of eight or ten sets of weighings was used, the maximum for both dry weight and ash was very close to 1 ppm. No such rise is shown in the repro-

duction constants in figure 3 and the average of eight or ten sets gave an almost horizontal line until close to 1 ppm. The influence of the manganese is therefore probably upon the assimilation of the plant and not upon the reproductive function, at least until the region of toxic concentration is reached.

It was possible to obtain more information concerning the effect on composition, by determining the approximate green weight of the plant. This weight was obtained by washing the fronds through two changes of distilled water, and removing the surface water by pressing lightly between filter papers. The plants were then placed in the platinum crucibles and these weighed inside the closed weighing bottles, as described for the dry weight determination. The green weight showed a close parallel to the dry weight. The difference between the green and dry weights gives a fairly good approximation to the water content, and the volatile matter on ignition is easily obtained. When these were graphed on the logarithm of the concentration of manganese it was seen that the ratio of water, volatile matter and ash varied very little up to 1 ppm. of manganese; on the average there was an indication of a slight tendency for the ash to increase and the water to decrease. From 1 ppm. up, there was the same irregularity with increasing concentration of manganese as shown in the dry weight and ash.

The lower limit of the toxic concentration of manganese under these conditions can be assumed to be somewhere near 1 ppm. Below this concentration the addition of the element to the inorganic salt solution increased both weight and size of plant. It did not, however, have any effect upon the rate of reproduction of the Lemna, neither was there any marked alteration in the ratio of water in the plant to volatile matter or ash. Above 1 ppm. of manganese, reproduction, dry weight, ash weight and composition were all irregular and toxicity increased rapidly with higher concentration.

With regard to the essential nature of manganese we can find no trace of that element in the ash of the plants grown in the inorganic medium unless manganese is added to the solution. The water, after the third distillation (in pyrex glass) is kept in pyrex containers and the plants are grown in pyrex flasks. Boron and silicon may be obtained from this glassware by the plant, and there is a chance that some manganese could be obtained in this way also, although no trace of manganese could be found by evaporating 20 liters of the water which had stood in the glass containers for several weeks; also the ash of the plants grown in the stock solution gave no signs of its presence.

There is some indication that the plant will adapt itself gradually to a concentration of manganese which proves injurious at first; but whether the effect of the manganese, or the element itself, can be carried over for several

generations after the plant goes back to a lower concentration or a manganese free solution, is as yet doubtful, although some results point to that conclusion.

Summary

1. *Lemna major* has been grown in a medium of inorganic salts with and without the addition of manganese.

2. There is no indication that manganese is an essential element in the nutrition of the plant.

3. The size and weight of the fronds increase up to about 1 ppm. of manganese, but the ratio of ash, volatile matter and water is little affected. There is no increase in the rate of reproduction.

4. Above 1 ppm. the concentration becomes gradually more toxic, and reproduction, size, weight and ash all become very irregular.

5. Indications point to the probability of the adaptation of the plant to manganese.

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A MICROELECTRODE FOR THE RAPID DETERMINATION OF THE HYDRION CONCENTRATION OF EXPRESSED JUICES FROM SMALL AMOUNTS OF PLANT TISSUE[1]

B. C. Brunstetter and C. A. Magoon

(with one figure)

Introduction

In 1928, while working with Doctor J. S. Caldwell, of the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. D. A., it became necessary to obtain accurate data on the hydrion concentration of the outer and inner wall, septa, placentae, and pulp surrounding the seeds, of individual tomato fruits at various stages of development. For very young fruits (less than 15 days after fertilization) the material available was obviously limited and called for some type of microelectrode. Since numerous determinations were required, and each determination had to be performed quickly to prevent or minimize possible changes in the acidity of the expressed juice of the fruit part, a rapid as well as accurate technique was essential.

To meet these needs, the excellent microelectrode devised by Bodine and Fink (**5**) was chosen. Their apparatus consists essentially of a small electrode vessel, terminating in a capillary tube, and mounted on a modified microscope stand in such a way that the electrode vessel may be lowered by the coarse adjustment of the microscope until the capillary tube, filled with the liquid to be examined, makes contact with a saturated KCl solution contained in a U-tube connected with a calomel electrode. A platinum electrode is mounted at the top of the stand in such a way that it may be readily lowered or raised in the gas-tight electrode vessel. A side-arm on the electrode vessel provides for the entrance of hydrogen.

The amount of liquid required for a determination depends on the degree of capillarity of the end of the electrode vessel. In the form used by Bodine and Fink, only 0.015–0.020 cc. is required: they found it possible to make five to six acidity determinations on the blood of a single grasshopper without killing the animal. The samples could be drawn into the electrode vessel without significant exposure to air.

Later Bodine (**4**) used a form of this electrode in which the capillary tube of the vessel was finer, reducing the capacity to 0.01 cc.; with it he

[1] The authors wish to acknowledge the expert services of Mr. Leonardo Testa, 3543 S St., N. W., Washington, D. C., who made the microelectrode described in this paper.

made determinations of the acidity of the contents of individual fertilized or unfertilized ova of the marine fish, *Fundulus heteroclitus*.

Description of modified electrode

Two changes have been made in this microelectrode which increase its efficiency, as far as its use with the expressed juices of plant parts is concerned. For filling the electrode vessel, BODINE and FINK relied on capillarity plus the effect of shutting off the flow of hydrogen, when the tip of the capillary met the liquid under investigation. This method is suitable when the amount of juice available is less than 0.1 cc. and when the juice is free of cellular débris. In expressed plant juices, however, there is usually more or less débris present which tends to clog the entrance of a fine capillary tube. Since a comparatively large diameter reduces the capillarity of the tip, another means of filling the end of the electrode vessel is necessary. Consequently, a side tube, equipped with a stopcock and a small rubber bulb, was fused on the electrode vessel opposite the hydrogen inlet. Compression of the bulb expels some of the hydrogen in the electrode vessel so that as the pressure on the bulb is released, liquid is drawn up into the capillary tube when its tip is dipped under the liquid being investigated. By this means the amount of juice drawn up into the vessel can be closely regulated. Moreover, washing the electrode vessel between determinations is very much facilitated.

In place of the vaseline seal used by BODINE and FINK, a mercury seal has been substituted. The sudden changes of pressure involved in filling and emptying the tip of the electrode vessel with plant juices, many of which are quite viscous, have made this modification necessary. The mercury seal holds in cases where a gas passage would be forced through a vaseline seal.

Figure 1 is a line drawing, one-half actual size, of the electrode mounted on a microscope stand modified by removing the substage condenser and cutting away the central portion of the tube. Electrode A is a mercury-filled glass tube in the end of which a straight platinum wire J is fused; it is centered by means of de Khotinsky cement in a small brass collar C, machined to screw into the milled brass top D which fits tightly into the upper end of the sawed-off microscope tube F. A one-hole rubber stopper G holds the upper end of electrode vessel Q in place. As the drawing shows, the tube A ends in a mercury seal MS. The side-arm O for filling the electrode vessel is equipped with a stopcock and a small rubber bulb P. The opposite side-arm N is the hydrogen inlet with a stopcock handily placed for controlling the gas flow. The central portion of the electrode vessel I is continuous with the upper tube Q, which has a small hole R, for the pur-

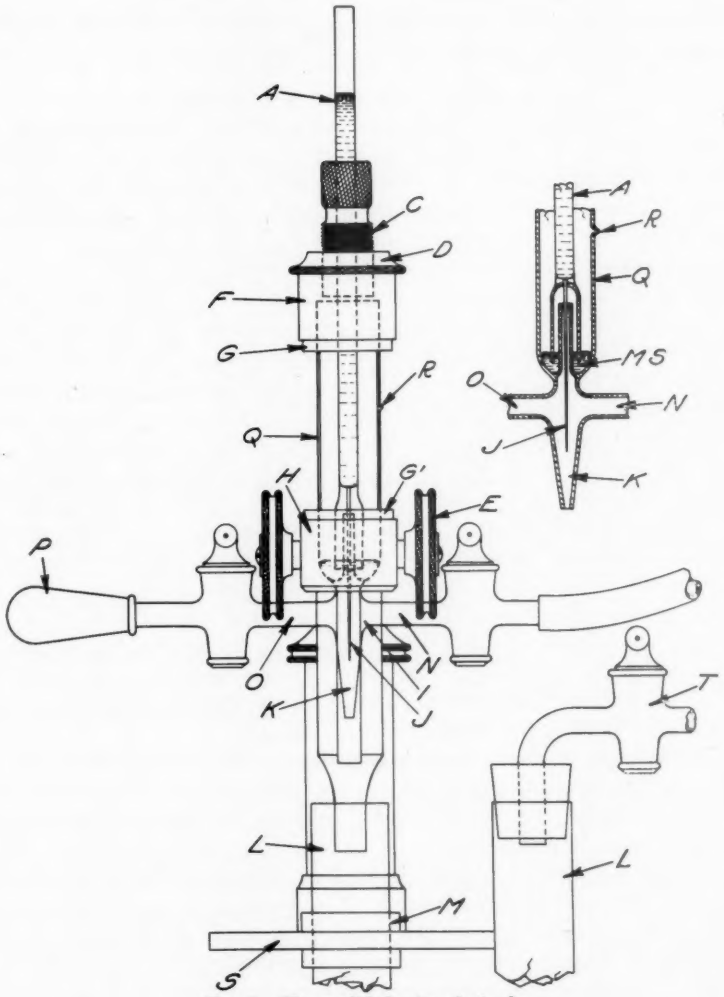

FIG. 1. The modified microelectrode.

pose of equalizing the pressure inside and out. The lower portion of the electrode vessel is held by means of the rubber stopper G′ in the lower end of the microscope tube H. By means of the coarse adjustment E the open capillary tip K can be raised from or lowered into the U-tube L which contains saturated KCl solution and connects with the colomel electrode T partly shown. The U-tube is held in the stage S of the microscope by means of the rubber stopper M.

Technique

The technique employed is as follows: The plant part to be analyzed is separated from the surrounding tissue and the juice is then expressed through cheesecloth into a small porcelain crucible. This is placed on top of one end of the U-tube and the electrode vessel which has been previously flushed out with hydrogen is lowered to make contact with the expressed juice. At the moment of contact the hydrogen is shut off and some hydrogen is expelled by compressing the bulb P. The pressure on P is then slowly released; when the rising liquid has reached the desired height, its ascent is checked by closing the stopcock on the arm O. This rinsing liquid is then expelled by hydrogen when the stopcock on the side-arm N is opened. After rerinsing, a sample is drawn up for analysis. The crucible is then removed and the electrode vessel is lowered by means of the coarse adjustment E to make contact with the saturated KCl solution. By means of the screw C the tip of the electrode is adjusted so that it just makes contact with the surface of the liquid. The potential is then read as quickly as possible by means of a suitable potentiometer.[1] Equilibrium is obtained in less than a half minute. (That equilibria reached so quickly are not "pseudo-equilibria" is shown (1) by the fact that a reading taken two or three minutes later, with the electrode just touching the surface of the liquid, agrees within two millivolts with the initial reading, and (2) by the excellent agreement of the initial reading of the microelectrode with a check reading by the Bailey electrode.) The electrode vessel is then lifted out of the saturated KCl solution and its contents are expelled into a small crucible by flushing the vessel out with hydrogen.

Washing the electrode vessel is performed in the same manner, and two rinsings with distilled water and one with the liquid to be analyzed may be quickly made. One determination followed by three rinsings requires on the average four minutes.

In working with plant juices it has been thought advisable for the sake of safety to replatinize frequently. The technique is quite simple. By unscrewing the collar C with the attached electrode A, the electrode may be lifted out entirely from the electrode vessel and cleaned by heating in a flame. (BIILMANN (**2**) advises against the use of a gas flame, and recommends the flame of either a benzene blow-torch or alcohol vapor lamp.) It is then returned to position and platinic chloride solution is drawn up into the electrode vessel, an accessory electrode is inserted into the platinic chloride solution in the crucible and replatinization is accomplished in the manner recommended by CLARK (**6**); a thin coating only is preferable. As a check on the nature of the coating, the electrode is tested with M/20

[1] In the work here described, a Leeds and Northrup Type K potentiometer was used.

potassium acid phthalate solution. As DRAVES and TARTAR (7) have shown, accurate readings may be obtained on this solution with the hydrogen electrode if its coating of platinum black is thin. A thick coating may result in upward drift of potential due, presumably, to reduction of the potassium acid phthalate.[2] After replatinization the vessel is thoroughly washed by rinsing at least four times. The whole process can be performed in less than five minutes.

Replatinization *in situ* is necessary in order to avoid the danger of scraping some platinum black off the electrode as it is inserted into the electrode vessel. If the end of the electrode vessel is drawn out into a fine capillary, the resistance of the platinic chloride solution becomes correspondingly great, so that a battery of sufficiently high voltage must be employed in order to replatinize readily.

This microelectrode can be used not only as a hydrogen, but as a quinhydrone electrode. The platinum electrode is cleaned by flaming it and then returned to position. Some quinhydrone is mixed with the expressed plant juice, the mixture is then drawn up into the electrode vessel so that the platinum electrode is deeply immersed. After making contact with the saturated KCl solution, the voltage is measured as usual. In this way the same piece of apparatus may be used to obtain duplicate readings by two different methods. It should be mentioned in passing, however, that the quinhydrone electrode is inapplicable to many plant juices, a rapid drift of potential occurring so that the pH calculated from a reading taken thirty seconds after mixing quinhydrone and juice differs materially from the pH obtained by the hydrogen electrode.

Discussion

In table I a few illustrations are given of some of the uses made of the modified microelectrode in this laboratory.

The acidities are given not only in terms of pH but also, to facilitate comparison and to obtain a mean with its probable error, as acidity units per liter, following the method proposed by WHERRY (**9, 10, 11**). One acidity unit is 1×10^{-7} gm. hydrogen ion per liter. To convert acidity units into hydrion concentration expressed as a normality, it is only necessary to point off the acidity units seven places to the left of the decimal place.

Determinations on the grapes, using the apparatus as a quinhydrone, then as a hydrogen, electrode, checked closely. The first determination on

[2] However, BLACKADDER (3) has obtained correct values (pH 3.97) on M/20 potassium acid phthalate solution using very heavily coated platinum electrodes. It seems that a thinly coated electrode invariably gives correct results tested on this standard but a heavily coated electrode may or may not do so.

TABLE I

ACIDITY VALUES, OBTAINED BY THE MODIFIED MICROELECTRODE, OF SOME EXPRESSED PLANT JUICES

MATERIAL	ACIDITY	
	pH	Acidity units[1] per liter
Grapes (Thompson's Seedless		
Mean acidity of series of 25 grapes, full ripe	3.84	1449 ± 41[2]
Acidity of juice from composite sample of 50 grapes (1)	3.83	1479
(2)	3.85	1413
Strawberries		
A. Diameter of berry = 13.0 × 11.0 mm.		
Taken from a sample at a stage following petal fall	3.42	3802
B. Diameter of berry = 15.0 × 14.0 mm.		
Taken from a sample in which the berries were making a rapid volume increase	3.03	9333
Tomatoes (Marglobe)		
A. Picked 12 days after fertilization.		
Weight = 11.61 g.		
Diameter = 29.6 × 24.8 mm.		
Wall	5.26	55
Septa and placentae	5.13	74
Pulp	4.79	162
B. Picked 28 days after fertilization.		
Weight = 28.58 g.		
Diameter = 41.3 × 34.4 mm.		
Wall	4.95	112
Septa	4.96	110
Placentae	4.80	159
Pulp	4.63	235

[1] cH is expressed, as recommended by WHERRY (9, 10, 11) as acidity units per liter, the unit being 10^{-7} gm. per L.

[2] Coefficient of variation: 21.34 per cent.

the composite sample of grapes was made by the former, the second by the latter, electrode. The figures on individual grapes were made by the quinhydrone electrode, but in all other analyses, the hydrogen electrode was used.

It is obvious that analyses of the acidities of an adequate number of individual fruits, instead of a composite sample of fruits has the advantage that not only the mean, but the distribution about the mean is obtained. Data of this kind are essential to throw light on the important question as

to what extent the acidity of a plant part at a given stage of development can be considered as a physiological constant.

The strawberries were a composite sample of several varieties of the everbearing type. This sample was picked and graded roughly into various stages of development by G. F. WALDO, of this Office. The data on strawberries and tomatoes, which are amply confirmed by other work not reported here, illustrate the marked increase in acidity which occurs in the early stages of growth of fruits.

The modified microelectrode has also been of considerable service in this laboratory in quickly checking the BAILEY (1) and the HILDEBRAND (8) types of electrodes. In this connection, it has been tried with satisfactory results on apples, peaches, tomatoes, and the leaves and the petioles of the sugar beet, the macro- and the microelectrodes generally agreeing within 0.05 pH.

To illustrate the value of the modified microelectrode in routine acidity analysis, where rapidity as well as an accuracy greater than that of the colorimetric method is desired, it may be mentioned that in one working day, 109 determinations of hydrion concentration of the juices of the wall and pulp of 51 tomatoes were made by the modified microelectrode, the samples being prepared by two assistants.

Summary

The microelectrode of BODINE and FINK is modified in two important respects to make it more suitable for dealing with plant juices: (1) a mercury seal is substituted for a vaseline seal and (2) filling and washing of the electrode vessel is secured by fusing a side-tube, equipped with stopcock and rubber bulb, opposite the hydrogen inlet. The electrode can function either as a hydrogen or as a quinhydrone electrode. The amount of liquid required for measurement depends on the size and shape of the electrode vessel and may vary from 0.25 to 0.01 cc. Data illustrative of the use of the microelectrode in plant physiological research are given.

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METHODS FOR THE QUANTITATIVE EXTRACTION AND SEPARATION OF THE PLASTID PIGMENTS OF TOBACCO

PAUL D. PETERSON[1]

Introduction

During the past year, in making pigment studies of several types of mosaic on tobacco, the writer has used rather extensively the methods of WILLSTÄTTER and STOLL as modified by SCHERTZ (**1**) for the extraction and separation of chlorophyll, carotin, and xanthophyll. The method has proved to be effective and sufficiently accurate for comparative studies of the mosaic diseases of tobacco. It has been possible, however, to make several short cuts in the method without the sacrifice of accuracy, and to eliminate, at the same time, the hazard of emulsion formation in the separation of carotin and xanthophyll. By making a slight change in the method it has been possible also to obtain and measure that portion of the green pigments which is in an altered condition in the plant tissues at the time of extraction. This fraction either has been lost in other methods of extraction and separation (**1**) or has been measured as part of the unaltered chlorophyll (**3**).

Materials and methods

The method is standardized on the basis of ten-gram samples of fresh leaf tissues, stems and petioles being excluded from the samples. With large leaves, such as those of tobacco, it is desirable also to exclude midribs and the large lateral veins. The leaves are thoroughly ground with quartz sand in a mortar and transferred with acetone to a Büchner funnel for subsequent extraction.

The pre-wash of the finely ground leaf tissues with 30 per cent. acetone is omitted. Most of the substances removed from the leaf solids by this treatment are insoluble in acetone of low water content and therefore are not extracted with the plastid pigments, but remain in the solids. The slight quantity present in the ether-acetone solution of the pigments is washed out by the first few water washes used to remove the acetone from the ether.

No sodium carbonate should be added to the leaf tissues at the time of grinding if it is desired to measure the altered chlorophyll fraction. The altered chlorophyll is soluble in dilute alkaline solutions and will be re-

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moved by the wash water used to wash the acetone from the ether-acetone solution of the pigments, if an excess of sodium carbonate is added to the leaf tissues at the time of grinding.

Both the grinding and the extraction of the sample are facilitated by the addition of acetone in 10-cc. quantities until a total of 50 cc. has been added. The addition of more than 50 cc. of pure acetone prior to the transfer of the sample to the Büchner funnel is of no advantage and is wasteful of solvents. The use of much less than 50 cc., however, is inadvisable because of the diluting effect of the water in the leaf tissues. If too little acetone is used a pasty mixture results which if drawn down on the filter with suction is difficult to extract. A more serious complication, however, is due to the fact that some of the substances in the leaf tissues, which are soluble in dilute acetone, are carried down into the suction flask and are thrown out of solution as a fine flocculate when more acetone is added. The presence of solids in the ether-acetone solution of the pigments and especially the presence of this fine flocculate increases the difficulty of washing out the acetone without the loss of some of the plastid pigments.

Redistilled acetone of low water content (85–95 per cent.) may be used instead of pure acetone to extract most of the plastid pigments, but enough should be added before transferring to the Büchner funnel to insure a concentration of at least 80 per cent. Pure acetone should always be used in the final washes, however, in order to dehydrate the solids before extraction with ethyl ether.

In order to obtain the altered chlorophyll fraction for estimation, the ether-acetone solution of the pigments is transferred to a 500-cc. separatory funnel and washed several times with pure distilled water to remove the acetone. The final washings should be free of flavones. The ether solution of the pigments is then washed very thoroughly with 50 cc. of dilute alcoholic KOH solution made by adding 10 cc. of methyl alcohol saturated with KOH to 1,000 cc. of water. Practically all of the altered fraction will be removed by the first wash with dilute KOH solution if the operation is properly performed. The layered solutions should be shaken together vigorously but for periods of short duration only. Prolonged violent agitation invariably results in the formation of emulsions. If emulsions form allow the solution to stand until both the aqueous and the ether layers have cleared. Finally, run the greenish KOH solution of the altered chlorophyll through a filter-paper into a 100-cc. volumetric flask. Wash the ether extract several times with small quantities of dilute KOH solution. Make the combined extracts up to volume with dilute KOH solution and estimate colorimetrically. This should be done very soon after extraction as the altered chlorophyll is very unstable. Keep the sample in a dark place until measured.

The altered chlorophyll fraction obtained by the above method from tobacco tissues is sufficiently free from other colored substances to permit its use in comparative studies.

Whether or not it is desired to measure the altered chlorophyll it should be removed prior to the saponification of the unaltered chlorophyll because of its effect in changing the color tone of the chlorophyllin solutions. It may be removed either with dilute KOH solution or with one per cent. sodium carbonate solution as recommended by SCHERTZ for the removal of the last traces of the flavones.

The amount of KOH necessary to saponify the chlorophyll is conditioned to a large extent by the volume of ether in which the pigments are dissolved. The greater the volume of ether the more KOH is needed to effect speedy saponification. The use of large quantities of KOH is to be avoided, however, because of the increased difficulty of extracting with ether the last traces of the yellow pigments from the strongly alkaline solution of chlorophyllin. In order to reduce the amount of KOH needed for saponification, ether should be used sparingly in the early stages of extraction. The amount used should not exceed 150 cc. Some of this will be lost in the process of washing out the acetone. A further reduction in the volume of ether may be effected by saponifying the chlorophyll directly in the separatory funnel instead of transferring it first to a bottle or flask. The above expedient eliminates, at the same time, several operations in the process of pigment separation.

After the removal of the chlorophyllin, the ether solution of carotin and xanthophyll should be thoroughly dehydrated by passing it through anhydrous sodium sulphate. Failure to dehydrate this solution before evaporating off the ether may result in the formation of emulsions when petroleum ether is added to redissolve the residue.

Transfer the residue from the distilling flask to a 500-cc. separatory funnel with a minimum quantity of petroleum ether and 75 cc. of 85 per cent. methyl alcohol.

Immerse the separatory funnel containing the petroleum ether-methyl alcohol fractions of the yellow pigments in a water bath at 45° C. and drive off the petroleum ether by means of a gentle stream of air. The force of the air should not be strong enough to ripple the surface of the liquid. When all but a thin layer of the petroleum ether has been evaporated off, remove the separatory funnel from the water bath and drive off the remaining petroleum ether while gently rotating the solutions in the funnel. The carotin will collect on the sides of the funnel and float on the surface of the methyl alcohol in thin flakes. Redissolve the carotin with 5 to 7 cc. of petroleum ether and drive off as above while gently rotating the solutions.

If the operation is properly performed none of the carotin flakes will be carried down into the methyl alcohol solution of the xanthophyll.

Run the methyl alcohol solution of xanthophyll through a tight filter paper into a 200-cc. volumetric flask. If the alcohol layer is removed slowly, the carotin flakes floating on the surface will collect on the walls of the funnel. The filter paper will catch the occasional small particle that may be carried down.

Dissolve the carotin in the separatory funnel with 5 cc. of petroleum ether. Add 50 cc. of 85 per cent. methyl alcohol by gently flowing it down the sides of the funnel. Without using the water bath drive off the petroleum ether layer with air while gently rotating the solutions in the funnel. Run off the methyl alcohol as before, filtering it directly into the 200-cc. volumetric flask. Repeat the above operation using 5 cc. of petroleum ether and 25 cc. of 85 per cent. methyl alcohol. Frequently the alcohol in this extraction remains clear or is only faintly tinted with yellow. In this case no further extractions are necessary. If the alcohol from this wash is distinctly yellow, however, repeat the last operation.

The combined methyl alcohol extracts of xanthophyll are made up to 200-cc. volume with 85 per cent. methyl alcohol for estimation colorimetrically.

The alcoholic solution of xanthophyll obtained by the above method of separation is always clear. There is no necessity of transferring the xanthophyll to ethyl ether for estimation. This latter step therefore is omitted.

The filter-paper used in filtering the xanthophyll solution is dried in front of a fan at room temperature without being removed from its funnel. The carotin is dissolved from the walls of the separatory funnel with petroleum ether and run through the dried filter into a 100-cc. volumetric flask. It is made up to 100-cc. volume with petroleum ether for estimation.

In the above method of separating the carotin and the xanthophyll the danger of emulsion formation is eliminated, and, as compared to other methods, the time necessary for making the separation is greatly reduced. The accuracy of the above methods compares favorably with the longer and more difficult method of WILLSTÄTTER and STOLL as modified by SCHERTZ. It is important, however, if accuracy is to be achieved by the new method, to keep the concentration of the methyl alcohol at or slightly below 85 per cent. Higher concentrations may remove some of the carotin with the xanthophyll. Concentrations of 90 per cent. or above dissolve carotin rather readily after the complete removal of the petroleum ether. When this happens the carotin fraction will be low and the xanthophyll fraction correspondingly high.

In a recent paper by SPRAGUE and SHIVE (**2**) the authors suggest the substitution of petroleum ether for diethyl ether as recommended by

SCHERTZ as a solvent for the carotinoids. The substitution eliminates the necessity of driving off the ethyl ether after the separation of the green from the yellow pigments. In the writer's experiments, however, this short cut has always resulted in low and erratic yields of xanthophyll. Part of the xanthophyll is carried down with the chlorophyllin. Repeated washings with petroleum ether fail to remove the xanthophyll although it can be extracted easily by a single wash with ethyl ether. Another objection to the use of petroleum ether is the increased difficulty of preventing the formation of emulsions thus making every step in the separation with petroleum ether slower than the corresponding step with ethyl ether.

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SOME GROWTH CURVES OF BARLEY KERNELS[1]

MARY L. MARTINI, H. V. HARLAN, AND MERRITT N. POPE

(WITH EIGHT FIGURES)

Introduction

In the years 1915 to 1920, Harlan, Anthony, and Pope conducted an extensive series of kernel studies, mostly at Aberdeen, Idaho. In these studies the daily growth was determined by weighing individual kernels from spikes of a definite age. The dry weights were obtained for the kernels from the same spikes. These results have been published in part only.[2] In the present paper the writers have made use of both published and unpublished material. It was thought that a series of fitted curves might present a trend of growth in a light sufficiently different from that of the previous treatments to justify its presentation.

Methods of procedure

The simplest logarithmic curve, $y = a + b (\log x)$, represents the general trend of most growth observations. A number of variants of this curve were tried in attempting to fit a curve to these data. The one finally used, $y = a + bx + cx^2 + d (\log x)$, gives an unusually good fit except for the first few days. For all varieties, with but one exception, Chevalier, this formula depresses the curve below the actual weights in these earlier days. In some instances this depression extends below the zero line.

It should be clearly understood that these curves are not prediction curves in any sense of the word. As stated above, these curves are roughly the growth curve common to all growing things. However, there are some very essential differences.

Up to the time of seed formation the barley plant is undergoing typical growth. Each day the added leaf and root tissue are functional and this accounts for the acceleration which is often likened to the acceleration of

[1] Contribution from the Office of Cereal Crops and Diseases, U. S. Department of Agriculture.

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compound interest or of catalytic activity. When seed formation starts, however, another factor must be considered. The addition of leaf and culm tissue diminishes rapidly and ceases entirely after a few days. From this time until near maturity the parts of the plant functional in producing elaborated food for storage do not increase. The seed theoretically is able to store the daily intake. Near maturity the leaves normally commence to mature and the functional parts thus undergo a daily reduction.

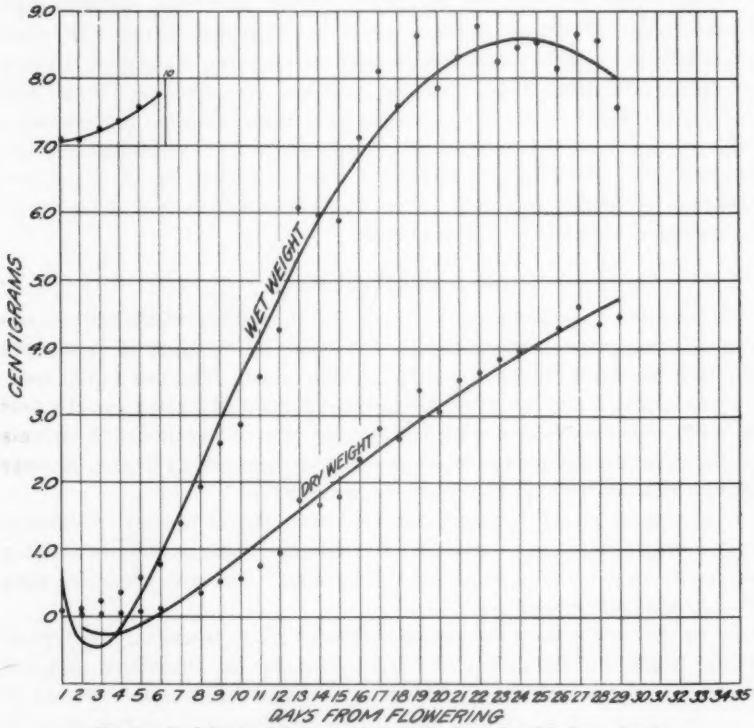

FIG. 1. Deficiens, with inset of first six days fitted independently.

The kernel itself, for a short period, undergoes development of a true growth nature. The cells of the endosperm are rapidly increased in number and the total rate of function of storage cells accelerated. By the time the growth of the culms and leaves is completed the number of storage cells has become nearly constant and from then until near maturity there is probably little change in the daily capacity of the kernels to store food, although some cells are added near the crease. During the main period of deposit

the dry matter curve is a nearly straight line. As maturity approaches there is less food to store, there are fewer cells of the kernel active, and, as far as these curves are concerned, there are individual kernels appearing in the averages which have ceased to function. Since the kernels mature one at a time, the acceleration in averages is modified considerably.

Discussion

The graphs tell the story of rapid or slow deposit as affected by variety more clearly than it can be told in words. It is unfortunate that the data on the hulled varieties could not be obtained for a longer period. The adherence of the hull after the kernel starts to dry makes this impossible. While the curves are not predicting curves, one can visualize by analogy with the naked varieties the probable after-trend in the hulled sorts. In the naked varieties there is no obstacle to prevent carrying the weights to full field dryness.

In figure 1 the growth curve of *deficiens* for 1918 is presented. The kernels of *deficiens* grow more rapidly and the final dry weight is greater than that of Hannchen which has been used in earlier publications. In this variety the formula used causes a very marked inaccuracy of fit in the first six days of the growth period. In both the wet and the dry weight the curve is depressed below zero and that of the wet weight depressed below that of the dry weight, although the kernels at this stage contain nearly 80 per cent. of water. This is purely a mathematical result. The curve of wet weight is more complicated than that of dry weight, as it contains one additional factor, water. If the same formula is used and the results based on the first 6 days only, this error is corrected. (See insert on figure 1.)

If the first 6 days are omitted in the wet weight, the fit for the succeeding period is much better than when the entire data are used. This period of *deficiens* is illustrated in figure 2. This can not but mean that the "S" curve of growth is not wholly applicable to the development of barley kernels. There evidently are more factors to be considered than mere growth. The lack of fit in the first 6 days is undoubtedly due to the fact that the kernel weights represent a number of factors. In the early stages when each cell of the endosperm and embryo is dividing, kernel growth acceleration probably is taking place. During this period the cells of the kernel probably are not capable of utilizing all of the food manufactured by the leaves and gathered by the roots and moreover some parts of the plant are still actively growing so that some of this food is being used elsewhere. Before the tenth day the food-gathering area and leaf surface have become stabilized and the entire output is deposited in the kernels. This results in a reasonably uniform daily deposit up to when signs of maturity appear.

As maturity approaches parts of the leaves cease to function and some of the cells in the kernels cease to be active in the deposition of starch. The final stages of maturity, as far as these curves are concerned, also are influenced by the fact that the kernels mature one at a time. Thus the averages on which the daily increment is based include an increasing number of kernels which have ceased to function.

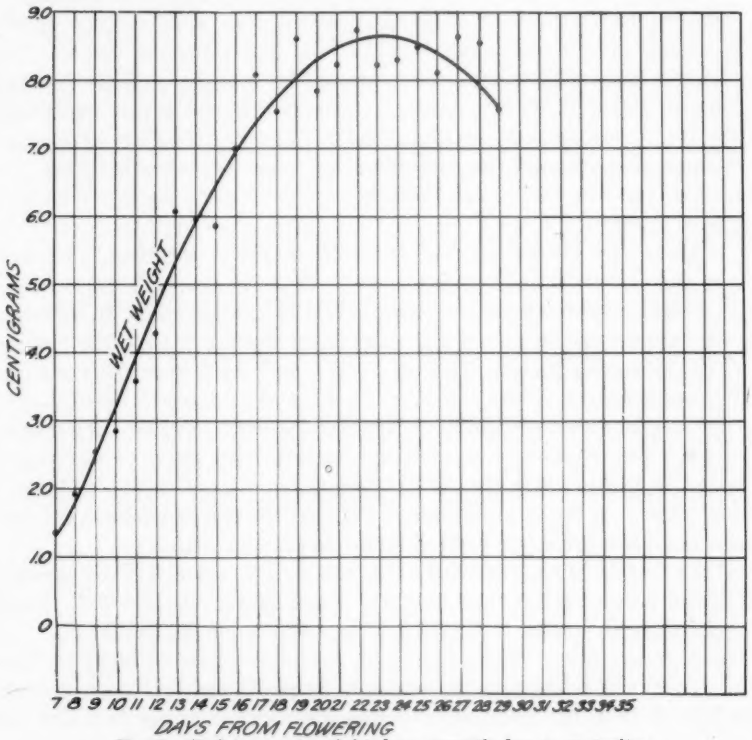

FIG. 2. Deficiens wet weight from seventh day to maturity.

This brings us back again to the formula. In order to secure a fit it was necessary to add another factor, C. What is the significance of C? Since it results in a fit of the data from 6 days to maturity, it must take care of a factor that is operating throughout this period. One significant and major factor in the growth of the kernel is the decrease of the percentage of water. This continues from the day of flowering until maturity, accelerating rapidly towards the end. Since the total water increases until near maturity and then falls rapidly, water might be considered first as a posi-

tive factor and then as a negative one. On the other hand, if the water increased proportionately with the dry matter, the daily growth would be much greater, and from this standpoint water acts as an inhibiter of full theoretical increment from the beginning. C is always minus in these data. It may, at least partially, thus take care of the difference between the 80 per cent. of water at flowering and the actual content on the successive days.

It is obvious that similar influences are operating in the curves of wet

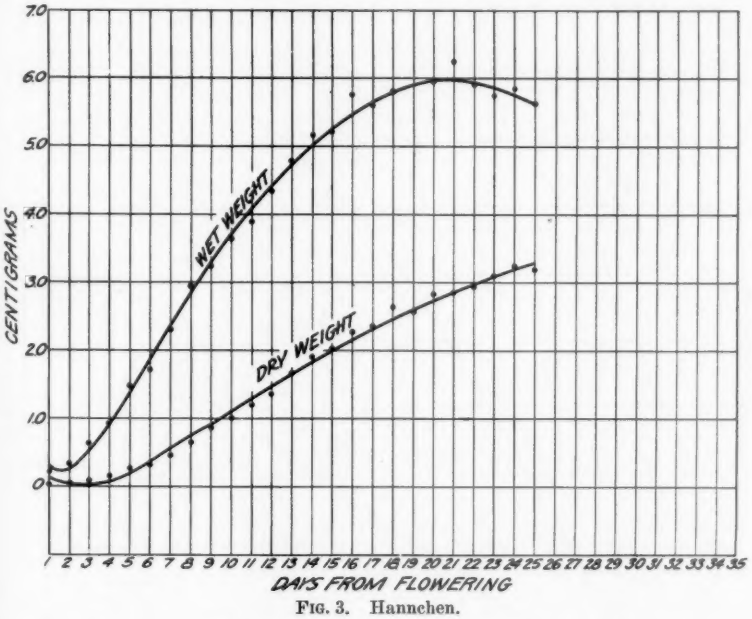

FIG. 3. Hannchen.

weight in other varieties and that they are susceptible of similar treatment. The date upon which the first curve should terminate varies, however, and it is thought that the date is more apparent to the reader if the curves are not altered.

Curves have been fitted to the data on 8 varieties. Figure 3 illustrates the results obtained from Hannchen in 1917. This variety is presented because it has been used in the earlier publications as a basis of kernel studies. It will be noted that the curve of the wet weight is still actively on the downward trend at the time the kernels had dried to the place where sampling was no longer possible. Hannchen is a small-kerneled variety as compared with other 2-rowed sorts and, indeed, as compared with the central kernels of the two 6-rowed sorts included in the 8 varieties.

In figure 4 the central kernels of Trebi barley were used as a basis. It is obvious that in this case the rate of deposit of dry matter had not yet been checked when sampling ceased. Indeed, the curve of wet weight had not yet turned downward. The large daily increment and the long continued activity doubtless are associated with the high yielding capacity of this variety. Even at the time sampling had ceased the dry weights were around 48 milligrams.

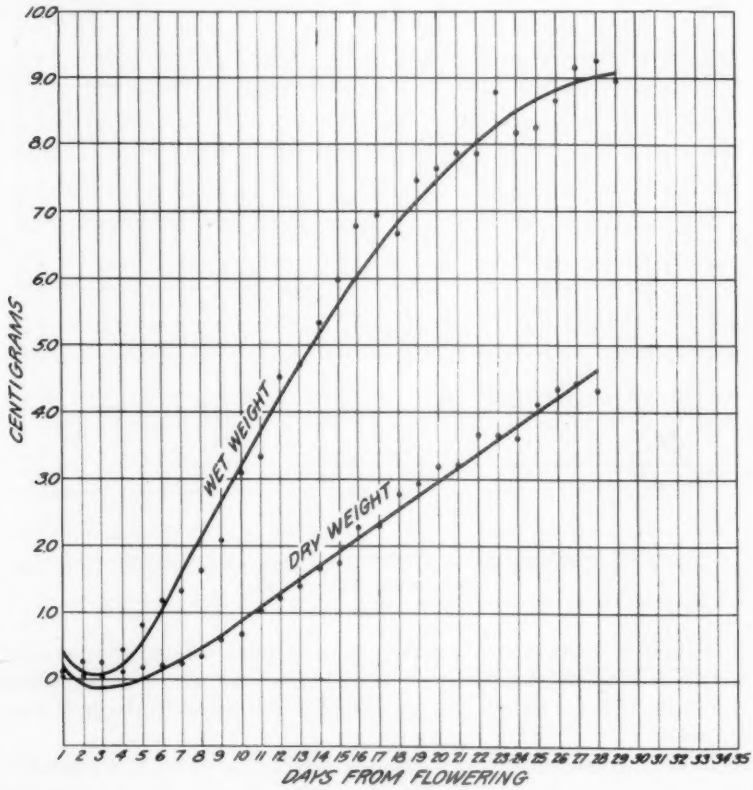

FIG. 4. Trebi.

Although there is no water in the dry weight, the formula containing C fits better than the same formula without it. The dry weights, however, are so nearly a straight line that no curve formula tried is wholly satisfactory with them.

Figure 5 represents the growth of Meloy, a hooded 6-rowed variety. Here again only the central kernels were used. The daily increase was

much less than in the case of Trebi and the curve of wet weight was markedly on the downward trend at the time sampling was stopped. The hooded varieties usually do not yield as well as the awned sorts, but Meloy is among the best of the hooded kinds. In most hooded sorts occasional kernels commence to ripen when most of the spike is still quite active. This, of course, would result in a lower average weight at full maturity.

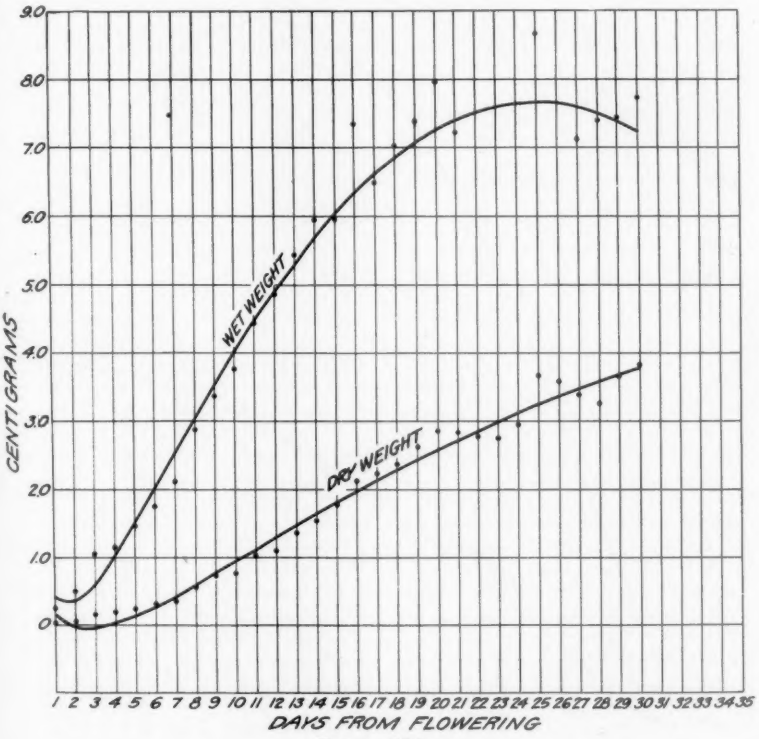

FIG. 5. Meloy.

Although naked varieties are not commercially important, they are well adapted to kernel studies. In the naked sorts sampling can be continued until the kernels are as dry as they will become in the field. A growth curve of Baku, a naked 2-rowed sort, is shown in figure 6. The original observations are included in the graph so as to show the relation of the fitted curve to the data. With the exception of the first days, the fit is all that could be desired. When sampling is continued to complete dryness, the wet and dry weights approach each other quite closely.

An interesting feature of figure 6 is the immense pyramid of water which is included in the actively growing kernels. Of course, the area between the curve of dry weight and that of wet weight represents water. This water, while possibly not truly functional, must be necessary for the accomplishment of the dry weight deposits.

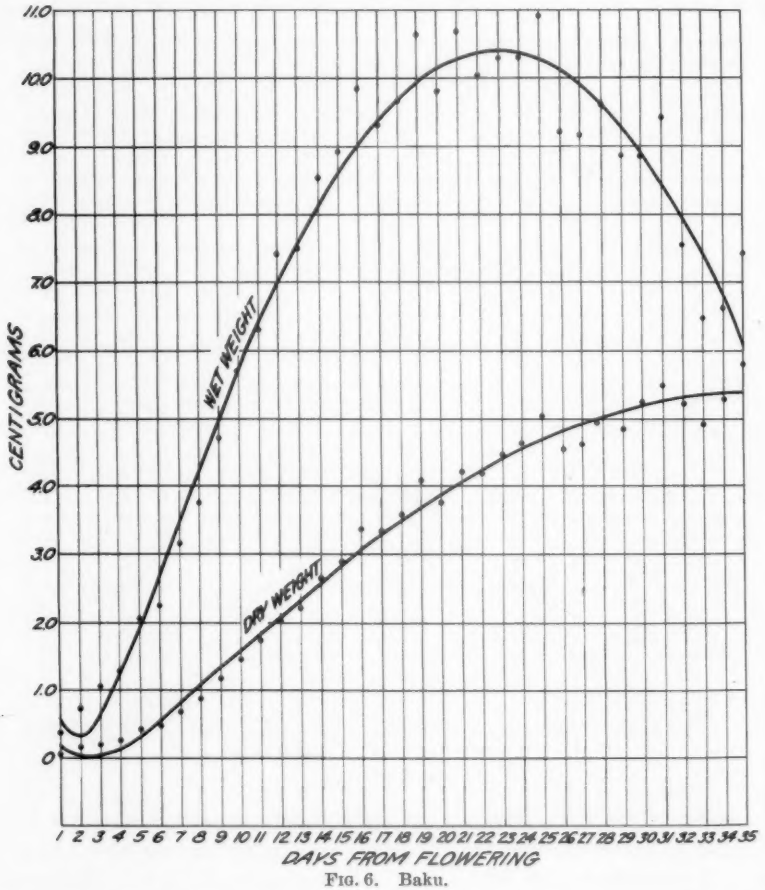

FIG. 6. Baku.

This period is even more striking in the curves of figure 7, which portrays the results obtained with Jet, another 2-rowed naked barley. The sharpness of the peak of wet weight in this case is due to two factors. The growing period is not so long as with Baku, which naturally results in a

more rapid decline after the peak is reached. The ratio of water to dry weight at the peak is a little higher in the case of Jet. This is probably due to the fact that this variety is possessed of a thickening of the tissue on the dorsal surface of the kernel outside the aleurone. This thickening runs lengthwise of the kernel directly opposite the crease. Its function is not known.

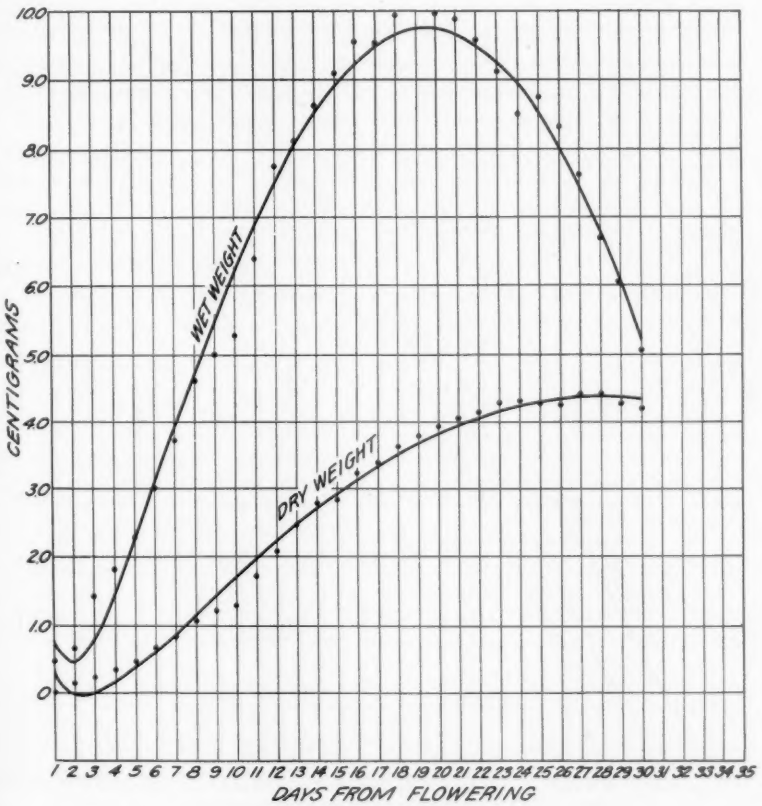

FIG. 7. Jet.

The wet weights of two 2-rowed varieties, White Smyrna and Chevalier, are given in figure 8. The dry weights of these varieties are not available. White Smyrna produces the largest kernels of any variety in our collection. The curve of wet weight is, therefore, steeply inclined and decidedly elevated at its peak. The full size of the kernel was not yet reached when sampling ceased.

In Chevalier the usual mathematical depression is not apparent at the beginning. The exact reason for this is not clear. Either the first samples were collected at a later stage than in the other varieties or they started growing with greater rapidity. It is likely that the kernels were older

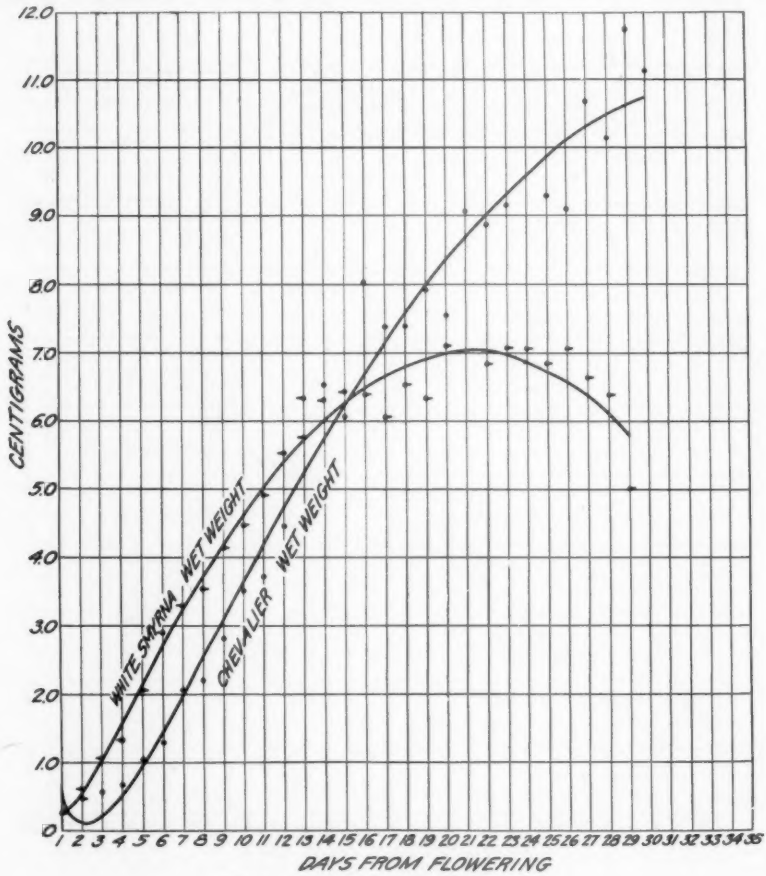

FIG. 8. White Smyrna and Chevalier.

when the sampling started. Spikes of identical age are most easily obtained by tagging them as the awns appear above the sheath. It may be that the spikes of Chevalier were more advanced than other varieties when the awns emerged and the time of flowering not accurately determined.

THE UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

A CASE OF SECOND CROP SEEDLESS APPLES[1]

C. B. WIGGANS

(WITH THREE FIGURES)

During the summer of 1925 a large number of second bloom apples were produced in the University of Arkansas Yellow Transparent orchard. Upon cutting some of these fruits in cross-section the writer was surprised to find them seedless. It seemed at first to be a case of parthenocarpy, but upon further study the evidence pointed to embryo abortion (**4**).

Mature apples normally have from a few to ten fully developed seeds. Various investigators have found a close correlation between the number of normal seeds and the setting and development of various fruits. SAX (**7**) found seed content to be correlated with setting and regularity in shape of apples, while MÜLLER-THURGAU (**6**) found size of grape berries to be closely related to seed content. The fact that lop-sided fruits are generally accompanied by the absence of seeds on the less developed side, as pointed out by

TABLE I

EFFECT OF SECOND BLOOM ON PERCENTAGE OF ABORTED OVULES IN YELLOW TRANSPARENT APPLES, 1925

DIAMETER SIZE OF FRUIT	NUMBER OF NORMAL OVULES	TOTAL FRUITS WITH ALL OVULES ABORTED	
mm.		*no.*	*per cent.*
7.5–10.0	0	11	100
10.0–12.5	0	47	100
12.5–15.0	0	40	100
15.0–20.0	2	56	97
20.0–25.0	0	72	100
25.0–30.0	1	36	97

WICKS (**10**) and others, is further evidence of the importance of normal seed development in connection with normal growth of the apple fruit. The cause of this uneven development of seeds may be due to improper pollination, abnormal ovules, unfavorable weather during or following pollination, or some other cause.

Although few of the fruits attained a greater size than 30 mm. in diameter and were, for the most part, malformed, many remained on the trees until frost. When over 250 of these fruits of various sizes were cut open, all were found to be without normal seeds except three. The three excep-

[1] Research paper no. 182, Journal Series, University of Arkansas.

tions had one normal seed each (table I). VINSON (8) reports a second crop of blossoms on Smokehouse, Rambo, and other varieties following frost destruction to the first crop but did not report on seed development in the second crop fruit.

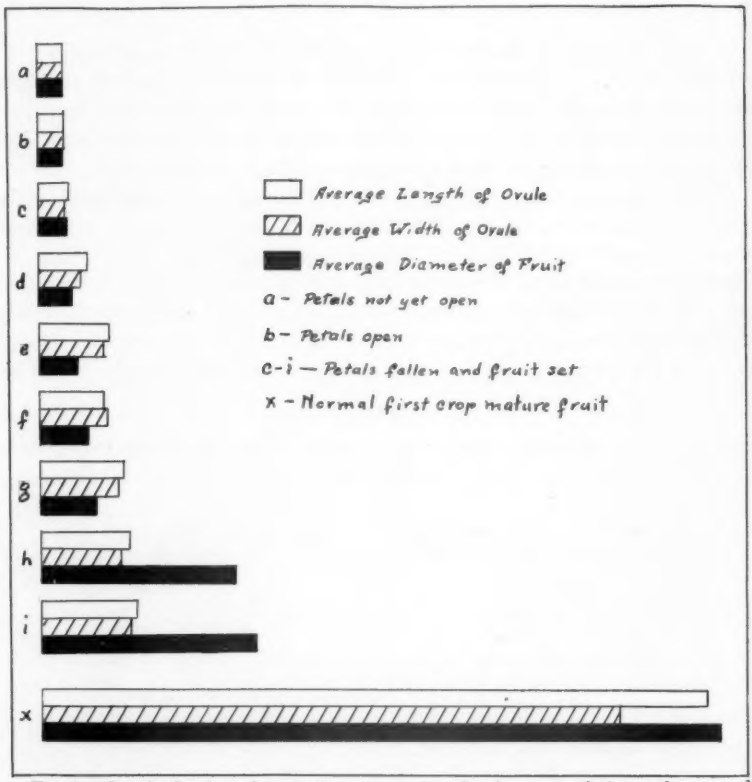

FIG. 1. Graph showing the relative progressive development of the ovules or undeveloped seeds and pericarp in second crop Yellow Transparent apples, and the approximate size of the same in normally developed first crop apples. All are expressed in percentage of size of specimen before blooming. Note the enlargement of ovules following blooming (e, f, and g), and the decided slowing up thereafter (h, and i).

The seedless condition, described above, was not parthenocarpic. This was borne out when, upon measuring the ovules and aborted immature or rudimentary seeds of the various sized fruits, it was found that there was a decided enlargement shortly following the fall of the petals. The apparently fertilized ovules rapidly increased in size, more rapidly than did the

pericarp for considerable time, but gradually ceased growing (table II and figure 1).

The ovules, under the microscope, appeared normal before petal fall, but shortly after that stage was reached they appeared shrunken and shriveled. Finally in fruits 30 mm. in diameter or larger, the ovules were brown and

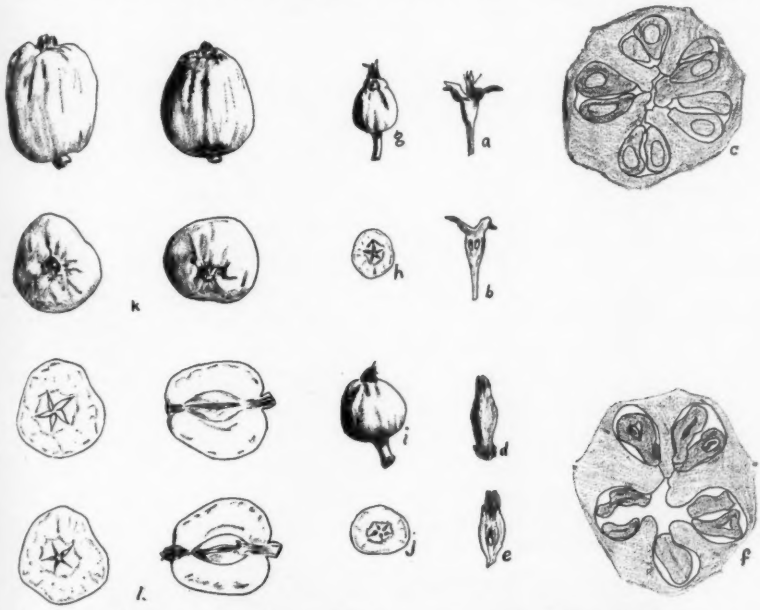

FIG. 2. Sketches of second crop Yellow Transparent at various stages of development.

A–B–C, immediately following petal fall showing ovules apparently normal.

D–E–F, a slightly later stage showing the somewhat larger, but already aborting embryos.

G–H, and I–J, successive stages showing the enlarging fruits with young seeds developed little, if any, more than in D, E, and F.

K, exterior appearance of seedless fruits.

L, the longitudinal and cross-sections of the same fruits as shown in K. Note the minute shriveled aborted seeds which have long since ceased to function.

were found with difficulty (figures 2 and 3). This is taken as proof that fertilization had been accomplished, but embryo abortion (**4**) occurred causing the seedless fruits.

By defoliating a Yellow Transparent and a Ben Davis tree in early summer, 1926, an attempt was made to force them into a second blossoming.

TABLE II

OVULE GROWTH COMPARED WITH FRUIT GROWTH, SECOND CROP YELLOW TRANSPARENT, 1925

STAGE OF DEVELOPMENT	FRUIT, AVERAGE DIAMETER	OVULES	
		LENGTH	WIDTH
	mm.	*mm.*	*mm.*
Petals not yet open	2.7	0.414	0.252
Flowers open	2.7	0.427	0.260
Petals fallen-fruit set	3.0	0.483	0.267
" " " "	3.5	0.791	0.418
" " " "	4.0	1.122	0.633
" " " "	5.0	1.050	0.668
" " " "	6.3	1.367	0.767
" " " "	20.0	1.435	0.792
" " " "	22.0	1.555	0.868

The Transparent tree responded rather well but not as well as expected. Only a few blossoms appeared on the Ben Davis. From this limited trial it seems that Transparent trees can be forced from the rest period more readily than the Ben Davis. The observations on the limited amount of material obtained in 1926 were substantially the same as in 1925.

In 1929 in spite of the extreme drought and the great loss of foliage due to disease early in the season little second bloom was observed in Northwest Arkansas and the writer was able to secure only four second crop fruits, and

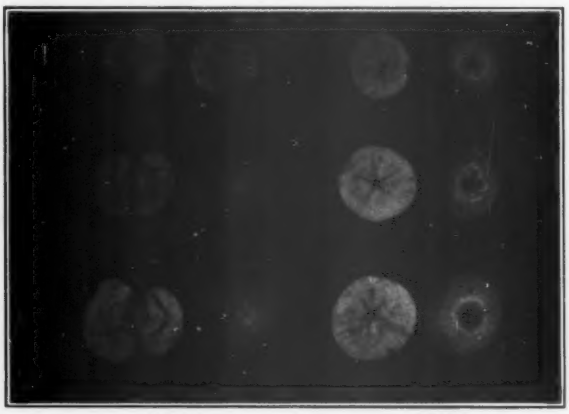

FIG. 3. Abnormal second crop Yellow Transparent apples. Only aborted seeds can be distinguished where mature seeds should be.

these were Coffelts. These fruits were one-half inch or less in diameter and were obtained after a severe frost had occurred so that the true condition of the young seeds could not be definitely established. Some of them appeared, however, to be more nearly normal than those described above. These ovules might have aborted at a later stage, although having bloomed under cooler weather condition, it is possible that they would have developed normally. It seems likely that the high temperatures during the second blossoming in 1925 and 1926 contributed largely to the abnormal development described.

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THE MASS FACTOR IN THE ENERGY RELATIONS OF LEAVES

CHARLES A. SHULL

In a recent paper the writer (**4**) emphasized the variability of the reflection factor in the energy relations of leaves, and pointed out the fact that reflection is frequently large, and not to be neglected in determining the absorption coefficient as was done by BROWN and ESCOMBE (**1**, pp. 75 and 91) in their fundamental studies of the energetics of the leaf. There is evident need of a reinvestigation of the problems of energy exchange between the leaf and its environment, and the internal transformations of energy in the processes of transpiration and photosynthesis, with due regard to the variability of the reflection and absorption of energy in all regions of the spectrum, from the far infra-red to the limits of the solar ultra-violet. Attention is called to an error in the previous paper (**4**, p. 604) which states that the BROWN and ESCOMBE studies were made "only on *Catalpa bignonioides.*" While some of their important measurements were made on the hypostomatous leaves of this species, in several sections of their work numerous species were used for studies of photosynthesis, thermal emissivity, and respiration.

The equation used by BROWN and ESCOMBE to calculate the heating effects of the absorbed radiation, on the supposition that there were no dissipation of energy by internal work, is $Ra/ms = t$ in degrees C. rise per minute, in which R is the total incident radiation per square cm. per minute, a the coefficient of absorption of energy, m the mass of the square centimeter of leaf lamina, and s the specific heat of the leaf substance. The values substituted for these symbols were as follows: $R = 0.8$ calorie, $a = 0.78$, $m = 0.020$ gm., and $s = 0.879$. The values used for m and s were obtained from measurements made on the leaves of *Helianthus annuus.* The value used for a is larger than that found for *H. annuus,* which was 0.687 as the average of three determinations, instead of 0.78; and the value assigned to R is also greater than those obtained during the work on *Helianthus,* which varied from 0.591 to 0.636 calorie per square cm. per minute, but less than the highest values observed on very clear days, which were from 0.932 to 1.019 calories. The authors state, however, that the mean results of observations extending over several hours were seldom over 0.550 calorie per square centimeter per minute, even on the clearest day. So the values assigned to the quantities in the numerator of the fraction in the equation are both relatively high.

It is obvious that the value of the denominator will vary either with the mass of the leaf per square centimeter, or with the specific heat. As most

leaves are fairly high in water content, the specific heat is probably much less variable than the mass factor. The latter is quite variable, and the value chosen by BROWN and ESCOMBE applies to a large group of thin-leaved plants, but does not apply very well to coarse textured or succulent leaves. In order to have a more general picture of the thermal effects of restricted internal dissipation of energy, some measurements of the mass factor have been made on a variable group of species which cover the range of possibilities pretty well.

If one uses the values assigned by BROWN and ESCOMBE to these various factors to calculate the rate of rise of temperature in a leaf in which no internal transformations of energy were occurring, the rise in temperature is about 35.5° C. per minute; but in the case of the heavy textured leaves, and especially with the succulent leaves, the temperature rise with zero dissipation of absorbed energy would be much less. In the thinnest leaves it is considerably higher than their estimate.

The data obtained in these measurements are given in the accompanying table I, and the thermal effects of energy absorption without energy dispersal are given in the final column of the table. In making these calculations, BROWN and ESCOMBE's values for R, a, and s have been used.

With only a few exceptions, the data of the middle column of the table are the average weights of 10 discs, each one square centimeter in area, the leaves having been cut with the Ganong leaf punch. In the exceptional cases the tissues were too thick for the punch, and pieces 1 cm. wide and 10 cm. long were cut accurately and weighed. In all cases the weights given are for 1 square centimeter of leaf tissue.

The heaviest leaf used was that of the aloe, whose thickest portions in the middle of the leaf weighed 69 times as much per square centimeter as the lightest leaves used (*Galinsoga parviflora*). Correspondingly, the rise in temperature per minute, on the basis of absorption of energy without dispersal, is approximately 0.75° per minute for the *Aloe* leaf, as compared to 51.8° for the leaves of *Galinsoga*.

If the value of R is taken as 0.55 calorie, which seems nearer the actual average energy receipt by plant leaves, and if the coefficient of absorption is corrected for the reflection, which averages about 10 per cent., the value of Ra will be greatly reduced. In addition the value of Ra should be reduced by the amount of energy used in photosynthesis, to obtain the quantity of energy to be dissipated in transpiration and thermal emissivity. The absorption coefficient found for *Helianthus* was 0.68, including the reflection, so that a value of 0.58 might be considered as near to the actual absorption. With these two modifications in the values, and subtraction of the energy used in synthesis, the energy to be dissipated is found to be only a

TABLE I

THE MASS FACTOR AND THERMAL EFFECTS OF ABSORBED RADIANT ENERGY IN THE ABSENCE OF ENERGY DISPERSAL

SPECIES	MASS PER CM.^{2}	Ra/ms
	gm.	*degrees C.*
Galinsoga parviflora	0.0137	51.81
Sagittaria latifolia var.	0.0139	51.07
" " "	0.0193	36.78
Ambrosia trifida	0.0142	49.99
Viburnum opulis americanum	0.0146	48.62
Ailanthus glandulosa	0.0152	46.70
Ptelea trifoliata	0.0156	45.50
" "	0.0159	44.65
Catalpa bignonioides	0.0163	43.55
" "	0.0191	37.16
Sambucus canadensis	0.0167	42.51
Ulmus americana	0.0174	40.80
" "	0.0214	33.17
Panicum sp.	0.0177	40.11
Amaranthus retroflexus	0.0180	39.44
Cucurbita sp.	0.0181	39.22
Populus deltoides	0.0197	36.03
" "	0.0201	35.32
" "	0.0213	33.33
Taraxacum taraxacum	0.02016	35.21
Rhus cotina	0.02024	35.07
Helianthus annuus	0.0212	33.49
Clematis paniculata	0.0223	31.83
Vinca variegata	0.02596	27.35
Geranium sp.	0.02604	27.26
Ginkgo biloba	0.0268	26.49
" "	0.0278	25.53
Syringa vulgaris	0.0317	22.39
" "	0.0333	21.32
Impatiens balsamina	0.0379	18.73
Dioon spinulosum	0.0380	18.68
Ceratozamia mexicana	0.0407	17.44
Nelumbo lutea	0.0520	13.65
Iris germanica	0.0731	12.45
" "	0.0808	8.78
Agave americana	0.1524	4.66
" " (mid-rib)	0.8300	0.85
Crassula arborescens	0.3037	2.34
Aloe arborescens	0.9465	0.75

little over 50 per cent. of the value used in the calculations of table I. The rate of temperature rise for *Galinsoga* leaves would be only about 26° instead of 51.81° per minute, and for the *Aloe* leaf about 0.37° instead of 0.75° C.

The energy receipt, if dissipated only by thermal emissivity, would cause a rise in the leaf tissue of only 4.4°, $\left(\frac{0.55 \times 0.58}{2 \times 0.0366}\right)$, above the temperature of the surrounding air, according to the figures of BROWN and WILSON (**2**) for air flowing at a little over 5 miles per hour. The fact that much higher temperatures have been measured in the leaves of some succulents (**3**) is related to the lower transmission in such thick leaves, and to greater energy receipt from the sun in very arid regions. When thermal emissivity and transpiration work together in the dispersal of energy, the rise in temperature required to dissipate the entire receipt of radiant energy is considerably less, possibly not over half as much as when thermal emissivity works alone. In the work by BROWN and ESCOMBE (**1**, table VIII, pp. 100–103), the energy lost by thermal reradiation and convection was in some instances 5 or 6 times as great as that lost by water evaporation, while in other cases transpiration exceeded thermal emissivity as a means of energy dispersal by as great proportion. Averaging the results of 13 experiments by BROWN and ESCOMBE, transpiration is responsible for about 55 per cent. of the energy dispersal, thermal emissivity for about 45 per cent. It is fair to assume then, that under ordinary outdoor conditions, the rate of rise in temperature of leaves by direct insolation will be less than half of the values shown in the last column of table I, on the average, and the total rise relatively small as the transpiration becomes decidedly the predominating agent of energy loss.

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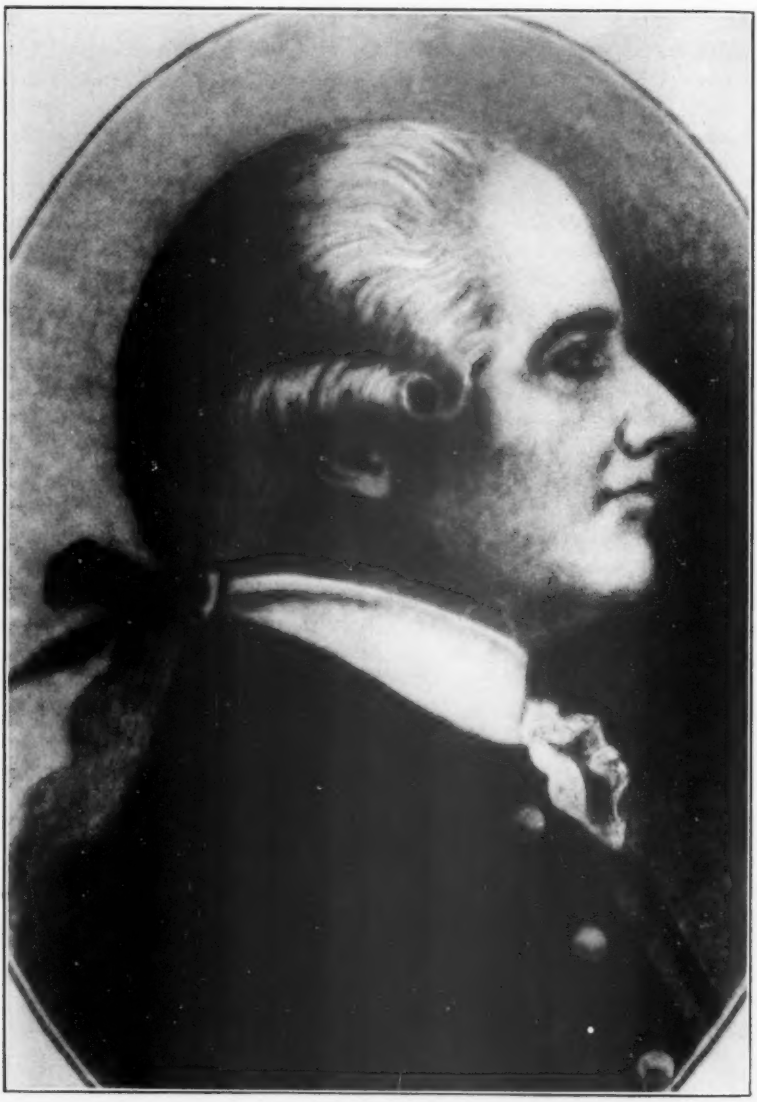


JAN INGEN-HOUSZ
1730–1799

UNIV. OF ILL.

BRIEF PAPER

JAN INGEN-HOUSZ

(WITH ONE PLATE AND TWO FIGURES)

JAN INGEN-HOUSZ (plate II) was primarily a physician, yet during the latter part of his life he became interested in the physiology of plants and made noteworthy contributions to this subject. His life and works have been extensively reviewed by Professor JULIUS WIESNER in a biography which was published at Vienna in 1905 under the title "JAN INGEN-HOUSZ, sein Leben und sein Wirken als Naturforscher und Artzt."

INGEN-HOUSZ was born December 8, 1730, in Breda, the head town of the province of Northern Brabant in the Netherlands. His father had come to Breda from Geldern, and JAN seems, therefore, not to have descended from the INGEN-HOUSZ family of North Brabant, famous in military affairs. The ancestry of JAN INGEN-HOUSZ is not known certainly beyond his grandfather.

Not much is known about the parents of INGEN-HOUSZ, but they had sufficient means and social position to give JAN an education far superior to that common to his time. His mother died when he was very young. He had a brother, LOUIS, who became an apothecary in Breda and from whom there descended a line of physicians.

At sixteen, INGEN-HOUSZ finished the Latin school in Breda, being remarkably proficient in Latin and Greek. The principal of the Breda gymnasium, HOOGEVEEN, prophesied a brilliant career for him.

He entered the University of Löwen to study medicine, and also became deeply interested in physics and chemistry. After receiving the degree of M.D. at the age of twenty-two, he went for further training to Leyden, to study under the famous teachers of medicine there, GAUBIUS and ALBINUS. After about two years there, he visited for a short time the Paris Medical School, and later the University of Edinburg.

In 1757 he came back to his home town of Breda and established a medical practice. He soon acquired a reputation for great ability, and his practice grew so large that it occupied all of his day time. He was obliged to carry on the scientific experiments in which he was so interested by night. He was particularly interested in frictional electricity, and perfected an electrical machine.

His successful medical practice and researches in physics built up a remarkable reputation for him in scientific circles. He kept up a widespread correspondence with his teachers in the universities which he had

attended, but was slow to publish the results of his experiments although his friends urged him to do so. This deliberation in publication is responsible for the maturity of his writings. His first publication appeared when he was twenty-eight years old.

The Netherlands could offer him no position suitable for his abilities, a teaching position in the University being closed to him on account of his religion. Ample opportunities were offered him in other countries, but love of his aging father kept him in Breda as long as his father lived.

After the death of his father, INGEN-HOUSZ accepted the invitation of Sir JOHN PRINGLE and other English friends to go to London. PRINGLE, then the foremost physician in England, was an old friend of the INGEN-HOUSZ family and had always been interested in the talented young physician. This interest led to a lifetime intimacy and mutual esteem. PRINGLE's influence opened the highest medical and scientific circles of London to INGEN-HOUSZ. He especially profited by association with the two HUNTERS, with ARMSTRONG, and MONRO.

INGEN-HOUSZ also made a visit to Edinburgh, where he made a special study of the clinical work of the hospitals. On coming back to London, he absorbed himself in the study of the diseases of children, particularly in the vaccination of children for smallpox.

At this time in London, also, he began to publish both medical and scientific papers. In several of these he advocated vaccination and attacked the opponents of it. He kept a small amount of time for the researches in physics, which he loved, and for association with famous physicists in London. But the major part of his time and attention he now determined to devote to the furthering of the cause of vaccination to combat the ravages of smallpox. It was his work in this subject that led to his call to the court of the Empress of Austria, Maria Theresa, to vaccinate her children.

INGEN-HOUSZ left London the first of April, 1768, made a short visit to his home in Breda, and arrived in Vienna on the 14th of May. He was received everywhere with honor, for his fame was widespread. In Vienna his countryman, GERHARD VAN SWIETEN, greeted him and the Empress had made his reception quite royal, having prepared for him two houses, one near the palace, and one in the country, at Schönbrunn. A coach and servants also awaited his pleasure.

INGEN-HOUSZ became a person of the greatest fame and popularity in Vienna as a result of his successful vaccination of the royal children. He was highly rewarded by the Emperor and appointed court physician, with a generous salary for the rest of his life.

In 1769 he journeyed to Florence, at the request of the Empress, to vaccinate the future Kaiser Leopold II. INGEN-HOUSZ undertook this mission

with great reluctance. The nervous strain of vaccinating so many prominent persons was telling upon him, and he wished to escape further honors and seek peace. This vaccination also was highly successful, to the great delight of the Empress. INGEN-HOUSZ lingered some time in Florence to regain his health and to visit with the physicist, ABBÉ FONTANA. This visit influenced his later researches on the gas exchange of plants.

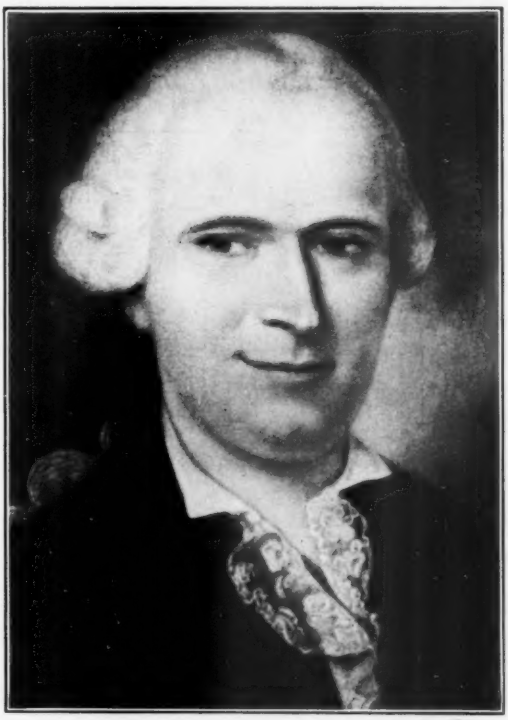


FIG. 1. INGEN-HOUSZ, from a contemporary pastel.

The appointment of INGEN-HOUSZ as court physician at Vienna had a great influence on his life, for it gave him the means and leisure to carry on the researches in science, in which he was so interested. After his return from a second Florentine trip until the end of his life he devoted himself to research. He desired to travel and visit with other scientists. He visited Switzerland in 1770, and the next year saw him in Paris, Holland, and England, then back to Vienna.

In 1775 he married AGATHA JACQUIN, the sister of the botanist, NICOLAS JACQUIN, professor of botany and chemistry at the University of Vienna.

Three years later found him again in England, where he began his research in plant physiology and physics and wrote his chief work: "Experiments upon Vegetables, discovering their great power of purifying the common air in the sunshine and of injuring it in the shade and at night."

Before going back to Vienna in 1780, he visited Paris, principally to make the acquaintance of BENJAMIN FRANKLIN, who was interested in a subject on which INGEN-HOUSZ later worked—the heat conduction of metals. He also visited FONTANA in Paris, being especially interested in the eudiometer method, which he found of the greatest use in his work on the gas exchange in plants.

FIG. 2. Bust of INGEN-HOUSZ in the Arcade at the University of Vienna.

INGEN-HOUSZ remained then eight years uninterruptedly in Vienna. To this period belongs his work on carbon dioxide assimilation in plants, in which he was assisted by Drs. MOLITOR, SCHERER, PICHEL, and his nephew, the younger JACQUIN. Most of his chemical and physical researches were carried on during this period in Vienna.

Kaiser JOSEF was very friendly with INGEN-HOUSZ and interested in his researches; he often visited his laboratory and watched him perform experiments. INGEN-HOUSZ's knowledge of electricity and magnetism was not exceeded by that of the famous members of the French Academy, BAILLY, GUILLOTIN, JUSSIEU, LAVOISIER, and LEROY.

In the summer of 1788 INGEN-HOUSZ again went to France. He arrived in Paris on the 14th of July, the day of the taking of the Bastille. This event upset his plans so that he decided to go to America to visit his friend, FRANKLIN. However, news reached him that his only brother had been

killed in an accident, which again changed his plans, and he started for Breda. The journey was very hard for him. He had to travel through Belgium, which had just freed itself from Austria. His position at the Austrian court made him very unpopular, and a number of unpleasant experiences marred his journey. He left Breda after a stay of one week and went to London. Here he learned of the death of FRANKLIN and gave up his American trip.

Depressed by the deaths of his two friends and exhausted by the trials of his journey, his health gave way. He took the advice of his English friends and decided to remain in England until political conditions in Europe warranted his attempting to journey back to Vienna in safety. But, as events turned out, he never again returned to Austria; for during the remaining eleven years of his life, conditions never prevailed in which he felt equal to attempting the journey back—much as he longed to go, as many letters to his wife and nephew indicate.

He remained in London, working or visiting his friend, DIMSDALE, at Herforth, or Lord LANSDOWNE, the statesman and patron of the arts and sciences. At Lord LANSDOWNE's estate at Bowood, his health improved and again he took up his researches on the nutrition of plants. Lord LANSDOWNE fitted up a laboratory for him. Here he wrote his second work: "An Essay on the Food of Plants and the Renovation of Soils." Agriculture of the Counties of Britain 14: 1–20, 1794–1795.

INGEN-HOUSZ died on September 7, 1799, after being in ill health for a considerable time. To the last, he hoped and planned to return to Austria, his adopted land. He was buried with great honors, in England, but the place of burial is not known with certainty. Fig. 1 is reproduced after a pastel made during his life, and fig. 2 shows the bust modeled by F. Seifert, which is now in the Arcade at the University of Vienna.—R. B. HARVEY, and HELEN M. WHITTIER HARVEY, *University of Minnesota.*

NOTES

Program Committee.—Active preparations are being made for the Cleveland meeting of the American Society of Plant Physiologists, in December 1930. The president of the Society, Dr. S. V. EATON, has appointed the program committee, as follows: Prof. J. H. GOURLEY, of the Ohio Agricultural Experiment Station and Ohio State University, chairman; Dr. JOHN W. SHIVE, of Rutgers University; Dr. W. J. HIMMEL, of the University of Nebraska; and Dr. A. R. DAVIS, of the University of California. The secretary-treasurer is *ex-officio* a member of the committee, also. The geographical distribution of this committee corresponds very well to the regional interests of the Society, and the chairman is located near the meeting place, so that arrangements can be given close personal attention. All of us can help the committee in its tasks by cooperating in any way that seems desirable. When papers are solicited for program purposes, a prompt and generous response would be greatly appreciated. Members may be called upon for other services, and the program committee should be able to command the active support of every member for that meeting.

Committee on Analytical Methods.—A slight change has occurred in the committee on methods of analysis. During the last several years Dr. W. E. TOTTINGHAM, of the Department of Agricultural Chemistry, the University of Wisconsin, has been chairman of this committee. At his request he has been relieved of the chairmanship, and Dr. J. J. WILLAMAN, head of the Department of Agricultural Chemistry of the New York Agricultural Experiment Station, has consented to become the leader of this important committee. Fortunately Dr. TOTTINGHAM remains a member of the committee which he has led so ably during the last several years. His service during this period deserves the thanks of all.

The de Vries and Ingen-Housz Portraits.—The portrait of DE VRIES in the January 1930 number of PLANT PHYSIOLOGY, and of INGEN-HOUSZ in this number, can be purchased from the editor of PLANT PHYSIOLOGY at 12 cents each. There are now six of these portraits of famous plant physiologists available, and the complete set can be obtained for office and laboratory use for 72 cents, postage paid. Postage stamps are accepted for all bills, and are preferred to checks from outside of Chicago, since the banks charge for collection on outside checks. The science of plant physiology has a fascinating and inspiring historical background. Developing students should have an opportunity to know about, and to appreciate the great scientists whose work forms the foundations upon which we are build-

ing today. Those desiring portraits should not wait until the earlier ones have been exhausted.

Stephen Hales Prize Fund.—The plans for enlargement of the STEPHEN HALES Prize Fund have been placed in the hands of a committee, consisting of Dr. R. P. MARSH, of Gettysburg College, and the Secretary-Treasurer. A letter has been sent out to the members, inviting cooperation in this enterprise. Would it not be a very appropriate thing to double the present fund? Every member of the Society has a right to feel proud of the fact that this fund has been established in honor of a great experimentalist, and that the fund will continue to stimulate and reward those who follow worthily in the footsteps of the forefathers of plant physiology. If each one will share in a small way in the privilege of contributing to the fund, the results will be gratifying to everybody.

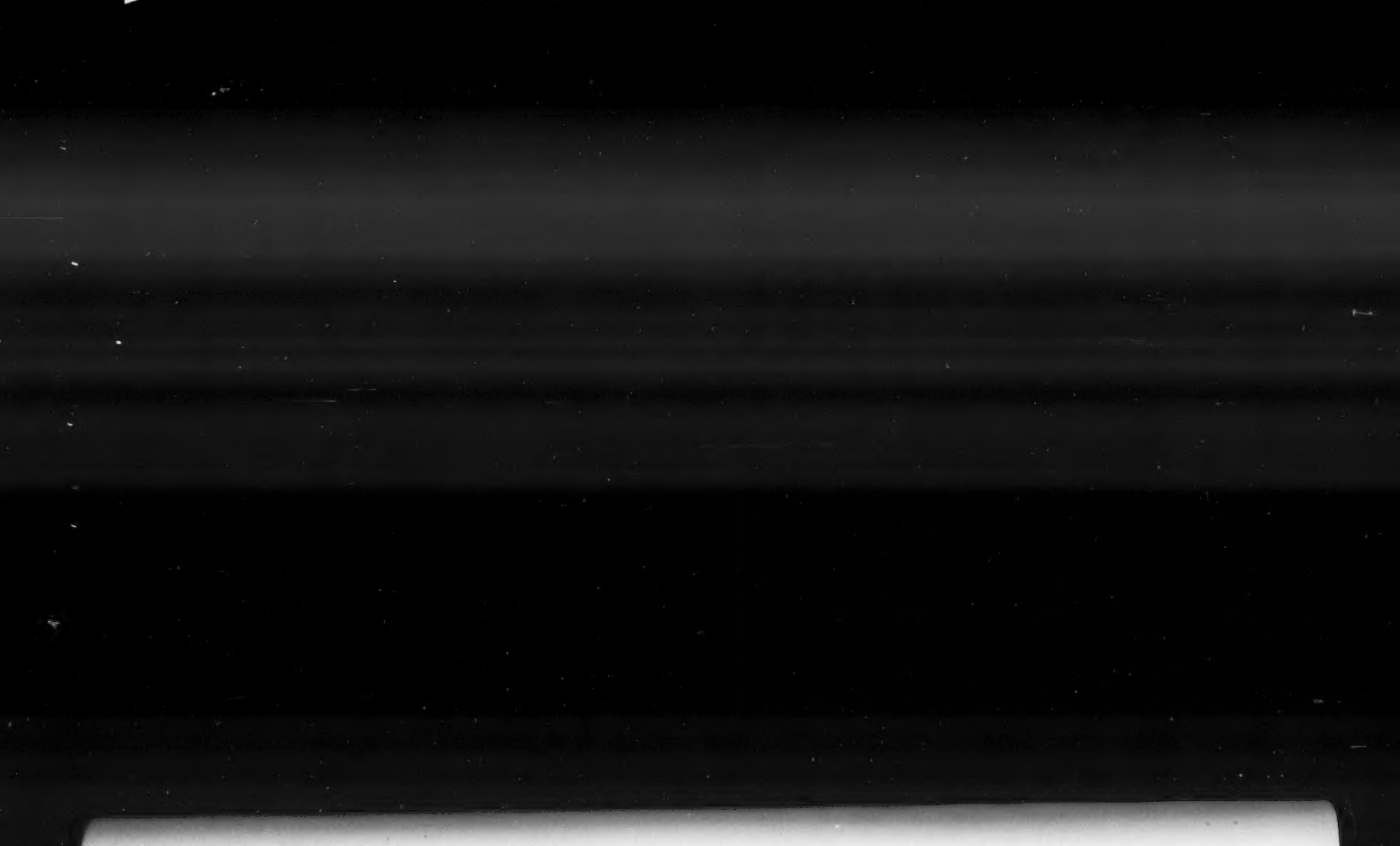
The Congresses.—Attention is called again to the Student Third Cabin Association which offers on the Holland-America Line attractive accommodations to anyone who plans to attend the international congresses which occur in Europe this summer. If you have not decided upon the manner in which you expect to reach these meetings, it would be profitable to investigate the S. T. C. A. way. An inquiry addressed to Mr. J. S. ROBBINS, passenger office of the Holland-America Line, 21–24 State St., New York, will bring full information about their accommodations, sailing dates, rates, etc.

International Address List.—The second bulletin of the American Society of Plant Physiologists, published in May 1924, was an international address list of plant physiologists. It proved to be a very valuable contribution. Our members will all be pleased to know that a second edition of this bulletin has been planned, to bring up to date this interesting and valuable list. The plant physiologists of the entire civilized world are figuratively "speaking the same language." There is something in the subject which draws us all together in bonds of friendship and fellowship. If the address list helps us all to know one another better, by facilitating exchange of ideas and helpful criticisms, and by promoting the solidarity of the group, it will serve a very useful function. Further announcements may be expected with reference to this bulletin.

Wild Flower Seeds.—If the seeds of western species of flowers are needed for research, it is possible to obtain seeds of many of the native species of California from Lester Rowntree and Co., of Carmel, California. Their recent list contains the names of several hundred different species, but prices are not quoted on many of them. Want lists would undoubtedly receive courteous attention.

Recent Advances in Plant Physiology.—A book reviewing the recent advances in plant physiology has been prepared by E. C. BARTON-WRIGHT, lecturer in King's College, University of London. While it is impossible to summarize all of the literature, the author has done well in his attempt to select the material in such a way as to mirror the advancement of the subject during the last 15 years. The order of presentation is logical, root and soil relations, transpiration and translocation, carbon and nitrogen metabolism, respiration, growth and development. The book will be especially useful to students who need a brief orientation to the recent progress. It is fortunate that some interest is being taken in the need for good works in this field. The price of the book is $3.50, and the publishers are P. Blakiston and Son, Philadelphia.

Hydrogen Ion Concentration in Plant Cells and Tissues.—The second volume of the Protoplasma Monographien from the press of the Gebrüder Borntraeger, of Berlin, is this work on Hydrogen Ion Concentration by Dr. JAMES SMALL, Professor of Botany in Queen's University of Belfast. There are 20 chapters, several appendices, and 25 pages of literature citations. The three sections into which the work is divided are the Introduction, presenting the problems, proteins, enzymes, buffers, sap, protoplast, and wall, and variation in reaction; Methods, including hydrogen electrode, quinhydrone electrode, micro-hydrogen electrodes, comparator indicator methods, capillator indicator, special indicator, range indicator, and buffer determinations; and Results, which gives a general survey of tissue reactions, diurnal and seasonal variation studies, and the data from individual plants, such as the sunflower, broad bean, potato, succulents, etc. The later chapters of the Results section take up the protoplast and pH, cell sap and pH, cell wall and pH, buffers and buffer indexes in plants. Chapter 20 restates the problems. It is a helpful summary in English of the whole field of hydrogen ion concentration. The price of the book bound in cloth is 30 RM. Orders should be sent to the publishers direct, W 35 Schöneberger Ufer 12 a, Berlin.

Laboratory Manual of Plant Physiology.—An attempt to revise the Detmer Praktikum having been made without success, Dr. L. BRAUNER, Privatdozent in the University of Jena, has prepared what is really a new book under the old title, Das kleine pflanzenphysiologische Praktikum. Part I, which is the only part yet received, deals with the chemistry and physics of the materials found in the plant body. Three other parts are projected, one dealing with the physical chemistry of the plant and its environment, another with the chemistry of metabolism, and a final section with structural modifications and movements. Part I contains 125 experiments,

covering the inorganic materials, and 13 groups of organic compounds. These experiments offer many suggestions for a successful course in the chemistry of plant physiology. In brochure form it is obtainable for 5.5 RM, and bound, for 7 RM. The publisher is Gustav Fischer, Jena, Germany. Plant physiologists engaged in laboratory teaching or investigation will find it a very useful guide.